# EARLY LIFE HISTORY OF PACIFIC HERRING: 1989 PRINCE WILLIAM SOUND HERRING LARVAE SURVEY

by

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## ABSTRACT

This study measured growth, mortality and dispersal of wild Pacific herring, Clupea harengus pallasi, larvae from two oiled sites and two non-oiled sites in Prince William Sound in order to determine the impact of the Exxon Valdez oil spill on larval population dynamics. Water temperatures and degree of stratification were highest in the two shallow control sites in the north of the Sound and lowest in the two oiled sites nearest Hinchinbrook Entrance. Concentration of prey of herring larvae increased over the May-June period and was not significantly different between sites. One major cohort and several minor cohorts of herring larvae were found at each of the four sites. No evidence was found to support the hypothesis that oil reduced growth rates or increased mortality rates of the larvae. Population growth rates measured from length frequency analysis ranged from 0.1 $mm \cdot d^{-1}$ in early May to 0.4 $mm \cdot d^{-1}$ in mid-June, and were highest in the two control sites and lowest in the two treatment sites. This was due to higher temperatures at the northern control sites, and not to any independent site effect. Recent growth rates measured by otolith ring widths rose from an average of 5.7% $\cdot d^{-1}$ in 10 d old larvae to 7.0% $\cdot d^{-1}$ in 30 d old larvae and then decreased to 5.6% $\cdot d^{-1}$ in 50 d old larvae; no differences were found between the four sites. Morphometric condition increased with age at a significantly slower rate at the Fairmount Island site than at the other three sites, but since it was a control site the cause was not related to oil. Mean RNA-DNA ratios of the larvae ranged from 4.65 at Rocky Bay to 6.39 at Tatitlek Narrows and did not vary significantly with site, date of capture or larval size and age. Mortality was highest at the Fairmount Bay control site, and lowest at the Rocky Bay treatment site. Transport of larvae away from the hatch site was greatest in the two southern treatment sites and least in the two northern control sites. This was most likely due to north-south transport of surface water in the Sound, and not to any oil-related factor. Comparison of densities of non-herring fish larvae between the four sites did not indicate any differences due to oil.

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## 1. INTRODUCTION

On March 24, 1989, the oil tanker Exxon Valdez struck Bligh Reef spilling 250,000 bbl of Prudhoe Bay oil into Prince William Sound, Alaska. Several weeks later, Triton Environmental Consultants Ltd. was hired by the U.S. National Oceanic and Atmospheric Administration (NOAA) to assess the impact of this spill on the growth and survival of Pacific herring, Clupea harengus pallasi, larvae hatching into the Sound. This is the final report of our investigations. It describes the population dynamics (growth, mortality and transport) of four cohorts of herring larvae that hatched into the Sound, and compares the dynamics between oil-contaminated and non-oil-contaminated sites. Appendices to this report are contained in a separate volume.

This study was planned in cooperation with the Alaska Department of Fish and Game (ADF&G) as one part of a seven part study of the impact of the oil spill on all life stages of herring in Prince William Sound. Commercial Fisheries Division of ADF&G is responsible for the first five components: (1) long term impacts on adult herring abundance; (2) impacts on adult herring growth and fecundity; (3) hydrocarbon content of adult herring tissues; (4) documentation of short-term adult herring mortality; and (5) field studies of herring egg survival. Triton is responsible for the last two tasks: (6) laboratory studies of herring egg and larval survival; and (7) field studies of herring larval growth, fitness and survival. Both studies (6) and (7) were administered under one contract. The results of the herring egg incubation experiment are described in a separate report(McGurk et. al. 1990).

## 2. STUDY SITES

The study was designed as a comparison between two oil-contaminated sites and two non-contaminated sites. Before proceeding further it is appropriate to define the labels we attach to the four sampling sites. At the time of writing we have no information on the concentrations of hydrocarbons that existed in the water column at the four plankton sampling stations in May-June 1989. Thus, we are forced to use a simple presence/absence index. This is complicated by the fact that most of the free-floating oil had left the Sound by April 4, almost one month before the start of hatching of herring eggs (Fig. 2). It is conceivable that few, if any, herring larvae were directly exposed to hydrocarbon concentrations above background levels.

However, to replace "oiled with the more vague label "treatment" is equally misleading because it implies a uniform and known level of contamination. In this report we use the words "oiled and "control" only because there are no better labels.

Selection of sites was based on a map of the 1989 herring spawn survey conducted by ADF&G, and on a map of the drift of oil produced by the Alaska Department of Environmental Conservation (ADEC). The spawn map showed that there were four major concentrations of spawning: the Northwest area centered on Tatitlek Narrows; the North area centered on Fairmount Bay; the Naked Island archipelago; and the northern tip of Montague Island. One minor concentration of spawn was observed in Sheep Bay. Fig. 1 shows the locations of spawn inside these five areas, the total length of spawn, and the range of spawning dates. Stars mark the locations of the plankton sampling stations.

The oil map, reproduced here as Fig. 2, showed that oil from Exxon Valdez drifted in a southeasterly direction from Bligh Reef through the Naked Island archipelago, down the west and east shores of Knight Island and out of the Sound through Montague Strait. Some oil drifted around the northern tip of Montague Island.

Therefore, the Northwest and North areas were chosen as control areas, and the Naked Island archipelago and the northern tip of Montague Island were treatment areas. The choice of sites within these areas was based on their proximity to beach transects from which herring eggs were removed for the herring egg incubation experiment, and on the depth of water. The sites had to be within 1 km of the beach transects in order to link the dynamics of the egg stage and the early yolk sac stage, as measured by the incubation experiment, with the dynamics of free-swimming herring larvae, as measured by the larval surveys. The water also had to be at least 30 m deep for the safe deployment of the bongo nets. Therefore, a site off the western shore of Fairmount Island (60°52.30'N, 147°28.80'W) was chosen because it was relatively deep and close to the transects inside Fairmount Bay (Fig. 1). Similar reasons guided the choice of a site inside Bass Harbor at Naked Island (60°37.40'N, 147°24.50'W) and a site inside Rocky Bay on Montague Island (60°21.25'N, 147°04.00'W). No beach transects were placed in Tatitlek Narrows, so the choice of a site in the northern entrance, off Black Point (60°54.40'N, 146°45.00'W), was based solely on depth concerns.

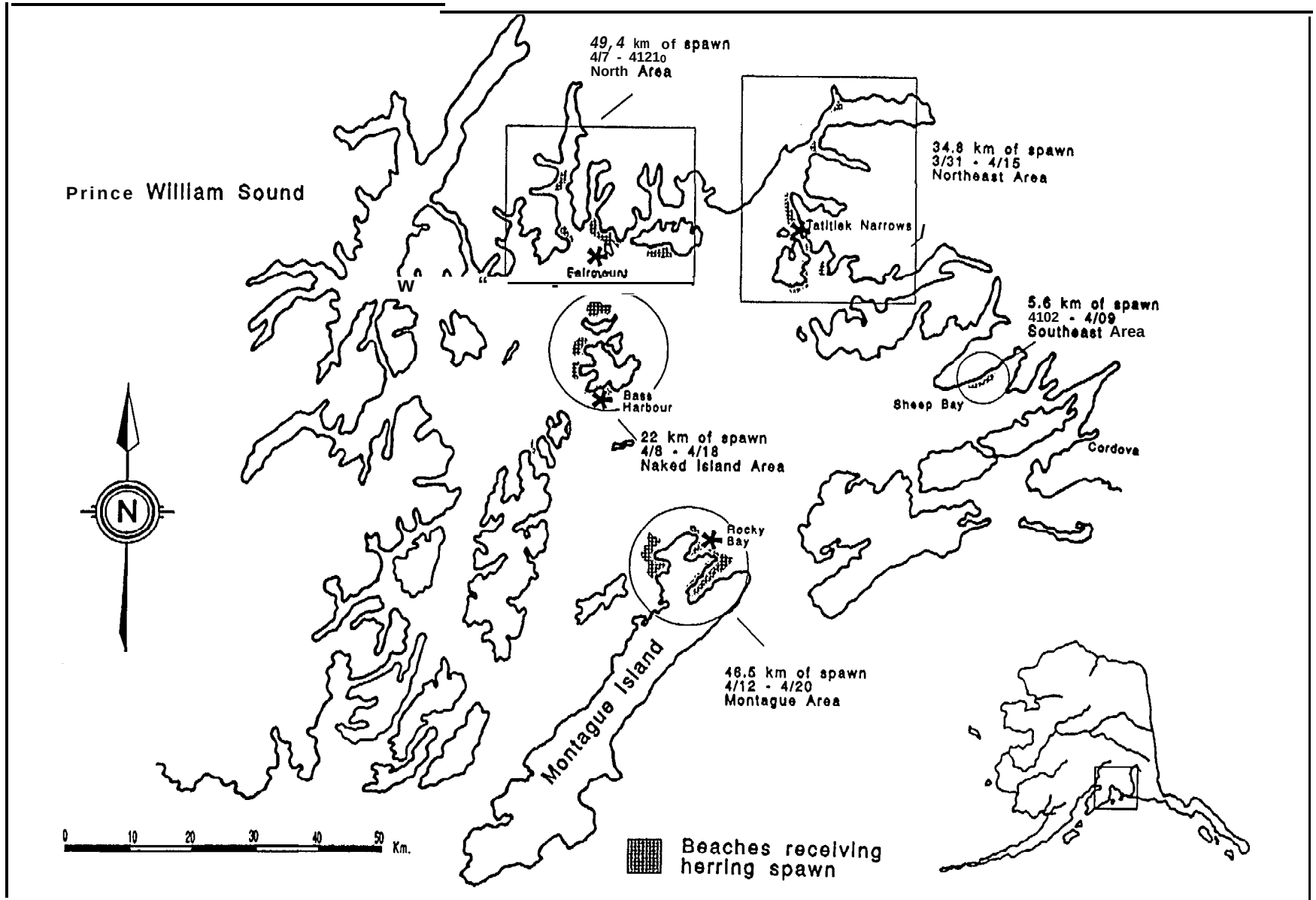


**Figure 1.** **Map of Prince William Sound showing the locations of herring spawning in 1989, the total length of spawn and the range of spawning dates. Stars mark the locations of the four plankton sampling sites.**

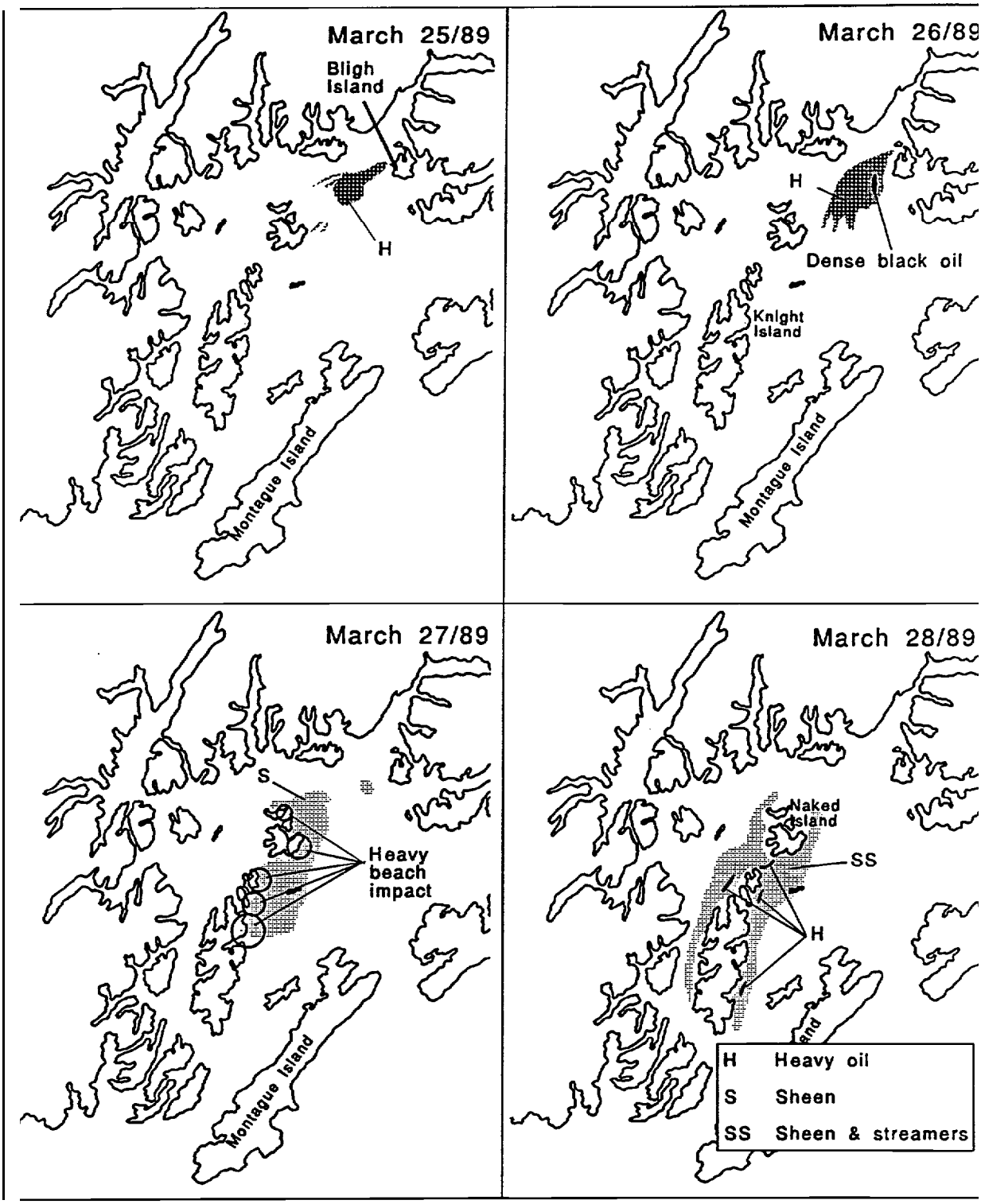


Figure 2. **Seven maps of Prince William Sound showing the trajectory of oil spilled from the Exxon Valdez from March 25 to April 4,1989.**

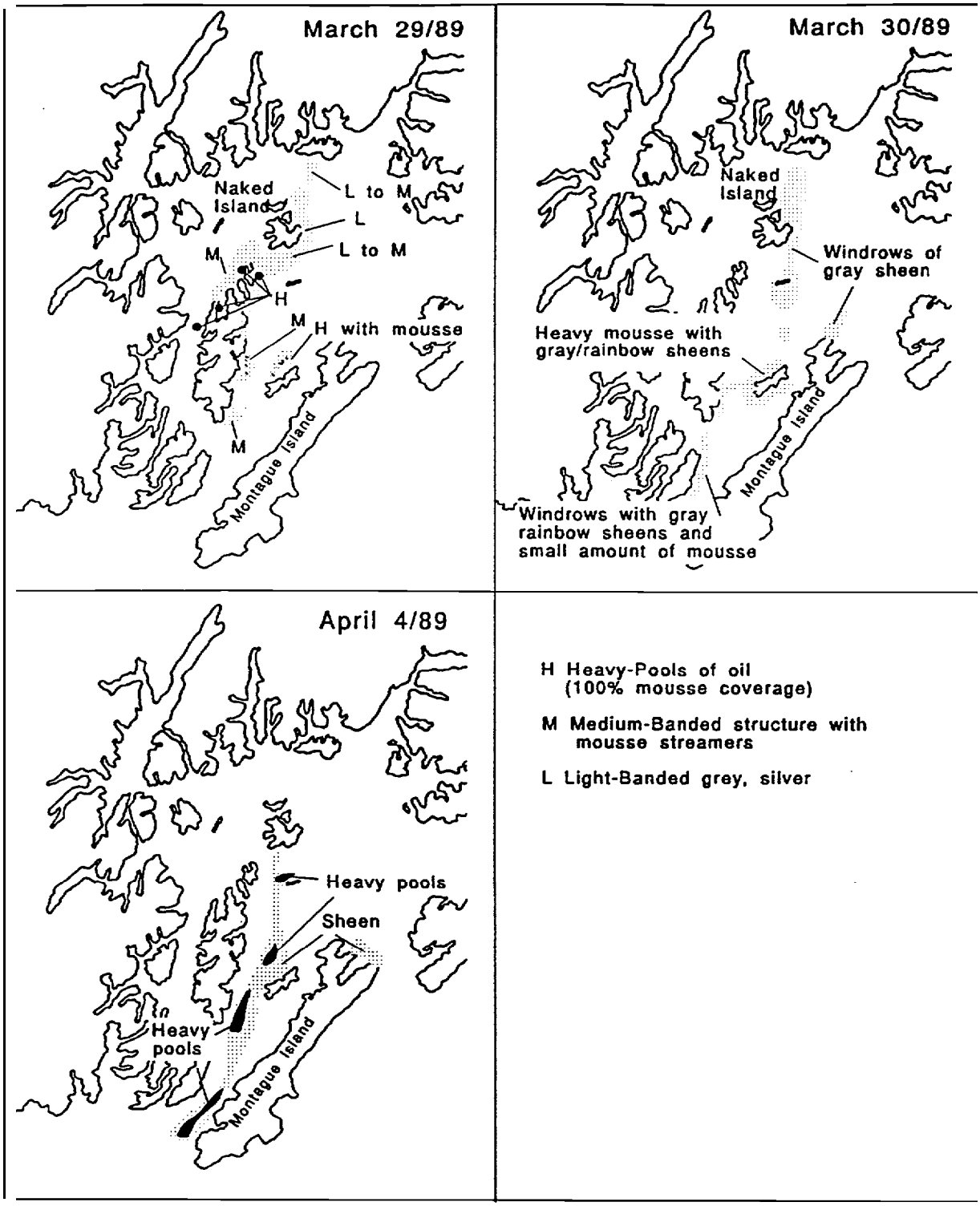


**Figure 2.** **Seven maps of Prince William Sound showing the trajectory of oil spilled from the Exxon Valdez from March 25 to April 4, 1989.**

## 3. MATERIALS AND METHODS

We conducted seven cruises of the Sound, one each week from May 1 to June 22, 1989. At each of the four sites, we collected samples of herring larvae for measurement of growth and condition and measured the density of herring larvae for estimation of mortality and transport. Auxillary information was also collected on the depth distribution of salinity and temperature, and on the densities of the zooplankton prey of herring larvae.

### 3.1 Plankton Sampling

The same sampling protocol was followed at each site. Temperature and salinity were measured at 2 m intervals from the surface to a depth of 30 m with a conductivity-temperature meter. These data are shown in Appendix D. Density of microzooplankton prey was measured by making four casts of a 30 L open-closing water bottle to 5, 10, 20 and 30 m, filtering the contents of the bottle through a 25 $\mu$m mesh bag and preserving the filtrates in 5% seawater formalin. The density of macrozooplankton was measured by towing a bongo net with a mouth diameter of 20 cm, a length of 1.5 m and a mesh size of 165 $\mu$m in a double oblique pattern from the surface to 30 m and back. The catch was preserved in 5% formalin. The volume of water filtered by all bongo nets was measured with a flowmeter placed off center in one of the two mouths.

Herring larvae were captured with double oblique tows to 30 m of a bongo net with a 60 cm mouth diameter, a length of 3 m and a mesh size of 333 or 505 $\mu$m. The 333 $\mu$m mesh was used to capture newly-hatched larvae and the 505pm mesh was used to capture older, mid-size larvae. The contents of the first 60 cm tow were preserved in 5% formalin for enumeration and morphometry. The contents of the second tow were preserved in isopropyl alcohol (37% by volume diluted with freshwater) for subsequent extraction of the sagittal otoliths. Finally, up to three more tows of the 60 cm net were done in order to capture live herring for RNA-DNA analysis. The live larvae were picked from the fresh zooplankton within minutes of capture and placed in small plastic vials which were immediately sandwiched between large blocks of dry ice (frozen $CO_2$: melting point of -56$^{o}$C) in an insulated chest. After the end of each cruise, the frozen larvae were flown or driven to Anchorage where they were stored at -70$^{o}$C in the freezers of the U.S. Fish and Wildlife Service. After

the end of the field season the frozen larvae were flown in blocks of dry ice to Microtek Research and Development Ltd. (Sidney, B.C.) where they were stored at -70°C until analysed for RNA and DNA concentration

### 3.2 Temperature and Salinity Contouring

In this report temperature and salinity are displayed in the form of depth-date contour plots in order to better show the timing of stratification and mixing events. The raw data used to produce these plots are contained in Appendix D. They were converted to contour plots using the computer program Surfer - version 4.0 produced by Golden Software, Inc., P. O. Box 281, Golden, Colorado. This program creates a regularly spaced grid using the inverse distance interpolation method:

$$(1)\quad Y = \left[\sum_{n} Y_i/(d_i)^2\right]/\left[\sum_{n} 1/(d_i)^2\right]$$

where Y = temperature or salinity at any intersection in the grid, Yi = neighboring point, di = distance of the neighboring point, and n = the number of neighboring points. This equation weights data points such that their influence declines with the square of their distance.

### 3.3 Prey Concentration

All micro- and macrozooplankton samples were identified and enumerated by Moira Galbraith of Sy-Tech Research Ltd. (Sidney, B.C.). Samples were first washed through a 40 $\mu$m seive in order to remove formalin. Very few organisms other than diatoms passed through this seive. The sample was scanned for exotic taxa which, if present, were removed for closer study. Then, the sample was split to a size of approximately 250 individuals and all were identified and enumerated to genus, species, sex and developmental stage. Copepod nauplii and copepodites were separated into four length categories: < 0.2 mm, 0.2-0.4 mm, 0.4-0.6 mm and >0.6 mm, before enumeration Minimum and maximum lengths and widths were measured for each taxon. Densities were calculated by dividing numbers by the volume of water filtered by each cast or tow. These densities are tabulated in Appendix E.

Animals smaller than about 0.4 mm in minimum length were extruded through the meshes of the 165 $\mu$m net (M. Galbraith, Sy-Tech Research Ltd., pers. comm.). Therefore, the densities of all organisms smaller than 0.4 mm were taken from the microzooplankton samples collected by the water bottle, and the densities of all zooplankters larger than 0.4 mm were taken from the macrozooplankton samples collected by the 165pm mesh net.

The list of organisms from both types of gear was first reduced by removing all organisms that are rarely eaten by herring larvae because they are too large to fit inside the mouth. Checkley's (1982) laboratory feeding experiments with Atlantic herring, Clupea harengus harengus, larvae showed that the maximum width of prey was 0.51 mm. McGurk's (1989b) review of the diet of Pacific herring larvae captured in the Strait of Georgia, showed that the maximum prey length was 1.4 mm. Other items were excluded on the basis that they have rarely ever been observed in the guts of wild herring larvae. This left a prey field consisting of microzooplankton: copepod nauplii, copepodites, bivalve veligers, gastropod veligers, trochophore larvae and small unidentified eggs; and macrozooplankton: 16 species of adult copepods, the cladoceran Evadne, and euphausiid nauplii.

A prey field was created from these remaining organisms using rules taken from McGurk (1989b):

maximum prey length = 0.050L - 0.095; and

minimum prey width = 0.019L -0.060.

where L = length (mm) of herring larvae. Prey concentrations were calculated from prey densities using weight-length relationships taken from the scientific literature:

copepods, cladocerans and euphausiid nauplii

Pearre (1980) reviewed weight-width relations of marine copepods to obtain

(2) $Y = 1.5598X^{2}*^{9776}$

### 3.5 Otolith Radius and Ring Number

Approximately ten herring larvae were randomly chosen from each alcohol-preserved sample and the two sagittal otoliths were removed from each larva with fine probes. They were prepared for examination with techniques described by Neilson and Geen (1980) and McGurk (1984a). Otoliths were examined under 400-1000X using a video camera and monitor attached to a compound microscope. Otolith radius was always measured along the longest axis because herring otoliths become increasingly ovoid as they grow larger. The number of rings was counted and the width of each of the outer five rings was measured. This data is shown in Appendix I.

### 3.6 RNA-DNA Analysis

RNA and DNA concentrations are shown in Appendix J. Details of the techniques used to measure RNA and DNA concentrations of herring larvae are contained in Appendix A. The method described by Clemmessen (1988) was found to be more accurate and precise than the methods of Karsten and Wollenberger (1972, 1977) and Bentle et al. (1981). All concentrations of nucleic acids measured by other methods were corrected to those expected from Clemmessen's (1988) method for non yolk sac larvae.

## 4. RESULTS

### 4.1 Temperature and Salinity

The isopleths of temperature and salinity shown in Fig. 3 show that temperature was consistently higher in the two control sites than in the two oil treatment sites, that salinity followed the opposite pattern, and that stratification of the water column began earlier in the two control sites.

These trends demonstrate the strong influences of average water depth, and of the distance between the sites and the entrance to the Gulf of Alaska. Both control sites were shallower than the two treatment sites and so they warmed up faster. They were also closer to the northern shore of the Sound and farther from Hinchinbrook Entrance. The northern shore is covered with glaciers that pump freshwater into the

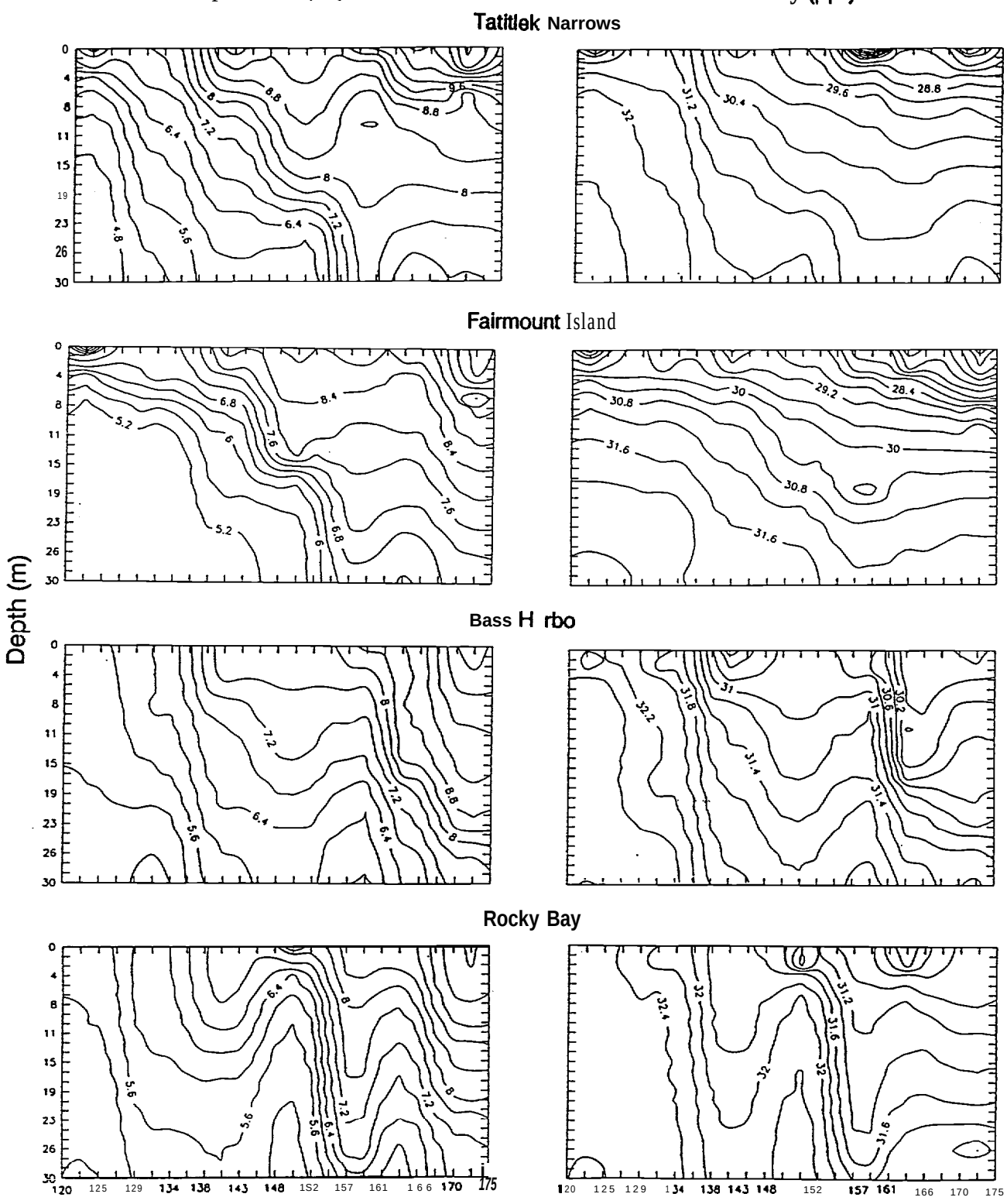


**Figure 3.** **Contour plots of temperature and salinity at four sites in the sound. See** text **for details of their construction. Depth profiles of temperature and salinity are shown in Appendix D.**

nearshore zone, but Hinchinbrook Entrance is the major site of entry for cold, high-salinity oceanic water from the Gulf of Alaska. Thus, the surface waters of the treatment sites are colder and more saline than those of the control sites.

Meunch and Schmidt (1975) and more recently Royer et al. (1990) have shown that the major inflow into Prince William Sound is through Hinchinbrook Entrance. At this channel the Alaska Coastal Current splits; a portion enters the Sound and the remainder proceeds westward along the southeastern shore of Montague Island. Within the Sound there is a cyclonic (counter-clockwise) circulation cell that is maintained by precipitation and runoff from the fjords along the rim of the Sound. In the northern Sound the flow is weak (e 10 $cm \cdot s^{-1}$) and westward, ultimately flowing southwestward down either Knight Island passage or Montague Strait.

### 4.2 Prey Concentration

Relationships between prey concentration, date of capture, and site of capture were analysed by, first, creating eight weight classes with equal size on a logarithmic scale:

| weight class ($\mu g$) | Contents |
|---|---|
| 0.10- 0.39 | small bivalve and gastropod veligers |
| 0.40- 3.19 | small copepod nauplii |
| 3.20- 6.39 | trochophore larvae, mid-size copepod nauplii, juvenile copepods |
| 6.40- 12.79 | large bivalve veligers and mid-size gastropod veligers |
| 12.80- 25.59 | large copepod nauplii, small and mid-size copepodites, juvenile and adult copepods |
| 25.60- 51.19 | large gastropod veligers, large copepoditeds, juvenile and adult copepods |
| 51.20- 102.39 | cladocerans |

Plankton biomass data is commonly ranked on a logarithmic scale for reasons of convenience in analysing the data, e.g. Sprules and Munawar (1986). Preliminary examination of the data showed that the distribution of mean concentration with weight had at least two modes and so it was not amenable to multiple regression

analysis, which requires smooth unidirectional changes in the response variable (Fig. 4). Therefore, a separate multiple regression was fit to each weight class. There were no significant (P> 0.05) differences between sites in prey concentration in any weight class, but there were significant (P = < 0.0001-0.015) positive increases in concentration with date in all weight classes except the two largest (Table 1). The fastest rates of increase were observed in the two smallest weight classes.

### 4.3 Number of Cohorts

The length frequency distributions of Fig. 5A-D show that one major and two minor cohorts of larvae hatched out at each of the four sites. Cohorts were numbered from 1 to 3 at each site depending on their estimated hatch dates; the earliest cohort was labelled number 1 and the latest cohort was labelled number 3. Some cohorts contained only one or two larvae. For example, cohort 1 of Fairmount Island (Fig. 5B) consisted of only one larvae captured on June 13, but this fish was much too large to be included in cohort 2, it was 9 mm longer than the next largest fish. Therefore, we assumed that it represented a very small cohort that hatched in mid- to late-April but was so small in number that it was sampled only once in seven cruises. The same reasoning applies to the two largest herring larvae captured at Rocky Bay on May 3 and May 12 (Fig. 5C); they were too large to belong to cohort 2 from this site so they were placed in their own cohort. Similarly, the two smallest larvae captured at Tatitlek Narrows on June 7 and June 12 were too small to belong to cohort 2 and so they were clasified as the sole representatives of a third cohort (Fig. 5D).

We assume that each of these 12 cohorts were separate entities and not mixtures of cohorts from other sites. We recognize that it is not possible to exclude mixing of cohorts because it is difficult to identify larvae from different areas by their mean length when these fish have hatched within one week of each other (Table 5). However, we believe it is a reasonable assumption because the catch curves shown in Fig. 15 do not exhibit the multiple modes that would be expected if cohorts mixed. Instead, the curves display a single mode (i.e. the dome of the catch curve) and the right-hand limbs of the curves display the steady decreases in density that are expected for single cohorts experiencing losses due to natural mortality and diffusion away from the centroid of distribution. This implies that the average rate of transport of larvae away from their hatch site and towards another sampling

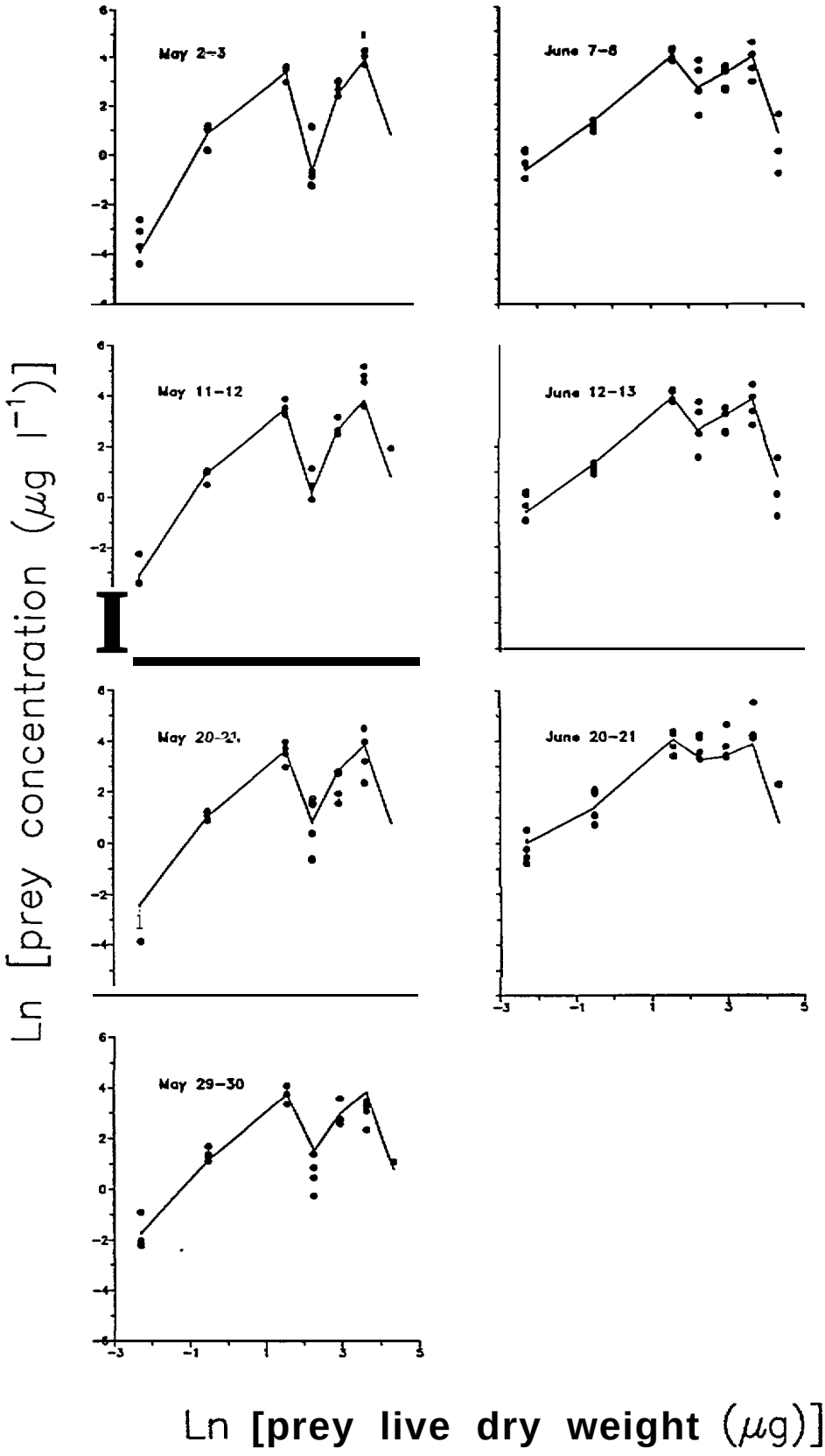


Figure 4, Observed (closed circles) and predicted (lines) concentrations of prey for **herring larvae plotted against weight class and date of capture. See Table lforregression equations. l'herewereno significant differences between sites in prey concentration.**

Table 1.

Regressions of ln[prey concentration ($\mu g \cdot L^{-1}$)] on Julian date for five weight classes of prey of herring larvae. Regressions for the two largest weight classes, 38.4 and 76.8 $\mu g$, were not significant.

| Midpoint of weight class ($\mu g$) | intercept | SE | slope | SE | $r^2$ | P |
|---|---|---|---|---|---|---|
| 0.1 | -13.6858 | 1.3027 | 0.0801 | 0.0087 | 0.76 | <0.0001 |
| 0.6 | -0.4881 | 0.6314 | 0.0110 | 0.0042 | 0.18 | <0.01 |
| 4.8 | 1.7079 | 0.5637 | 0.0136 | 0.038 | 0.31 | < 0.001 |
| 9.6 | -10.2971 | 1.5376 | 0.0791 | 0.0103 | 0.68 | <0.0001 |
| 19.2 | 0.2849 | 0.9011 | 0.0180 | 0.0060 | 0.22 | <0.001 |

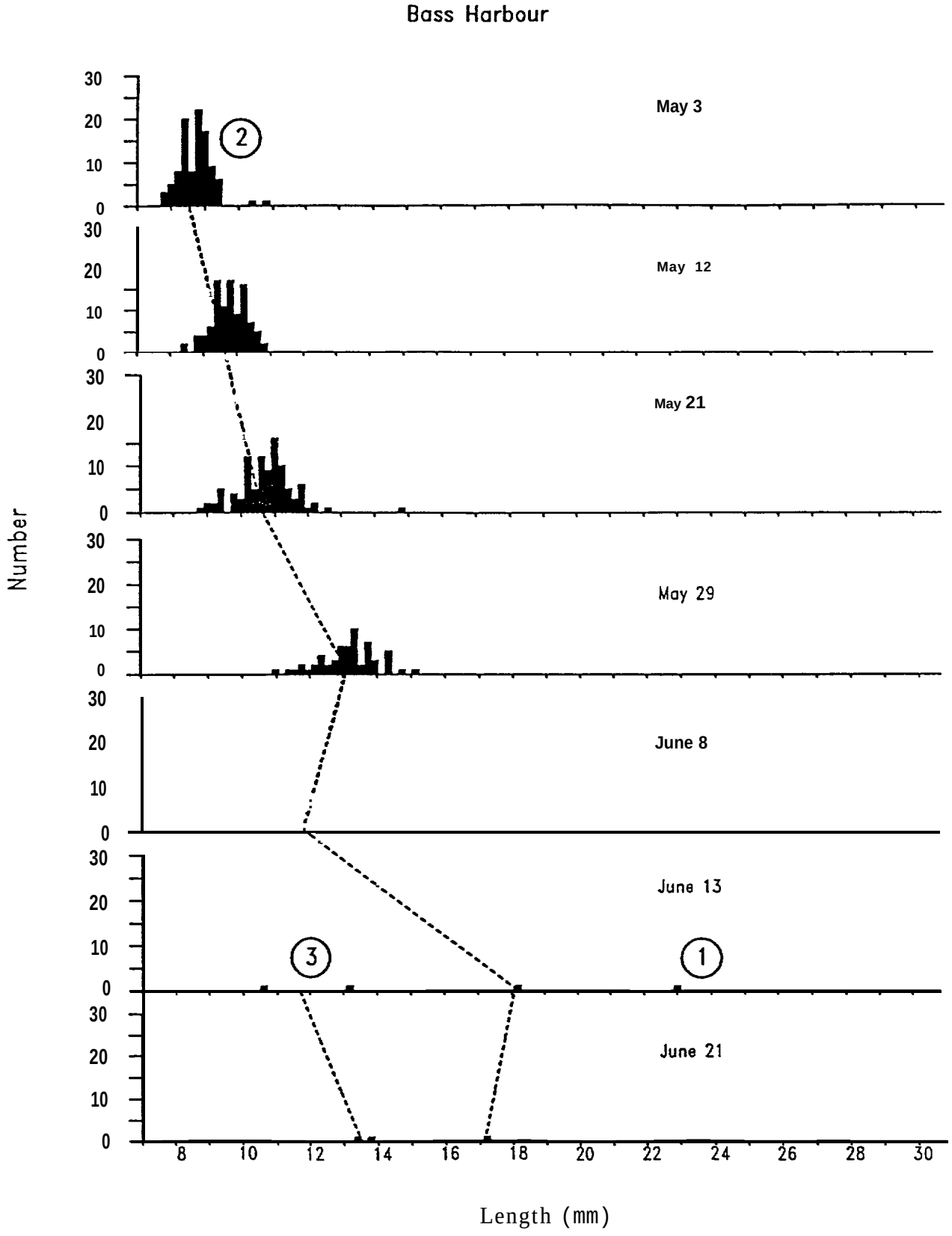


**Figure 5A.** **Length frequency distributions at date for the 3 cohorts of herring larvae found at Bass Harbor. Each cohort is identified by a circled number and by a broken line connecting mean lengths at date.**

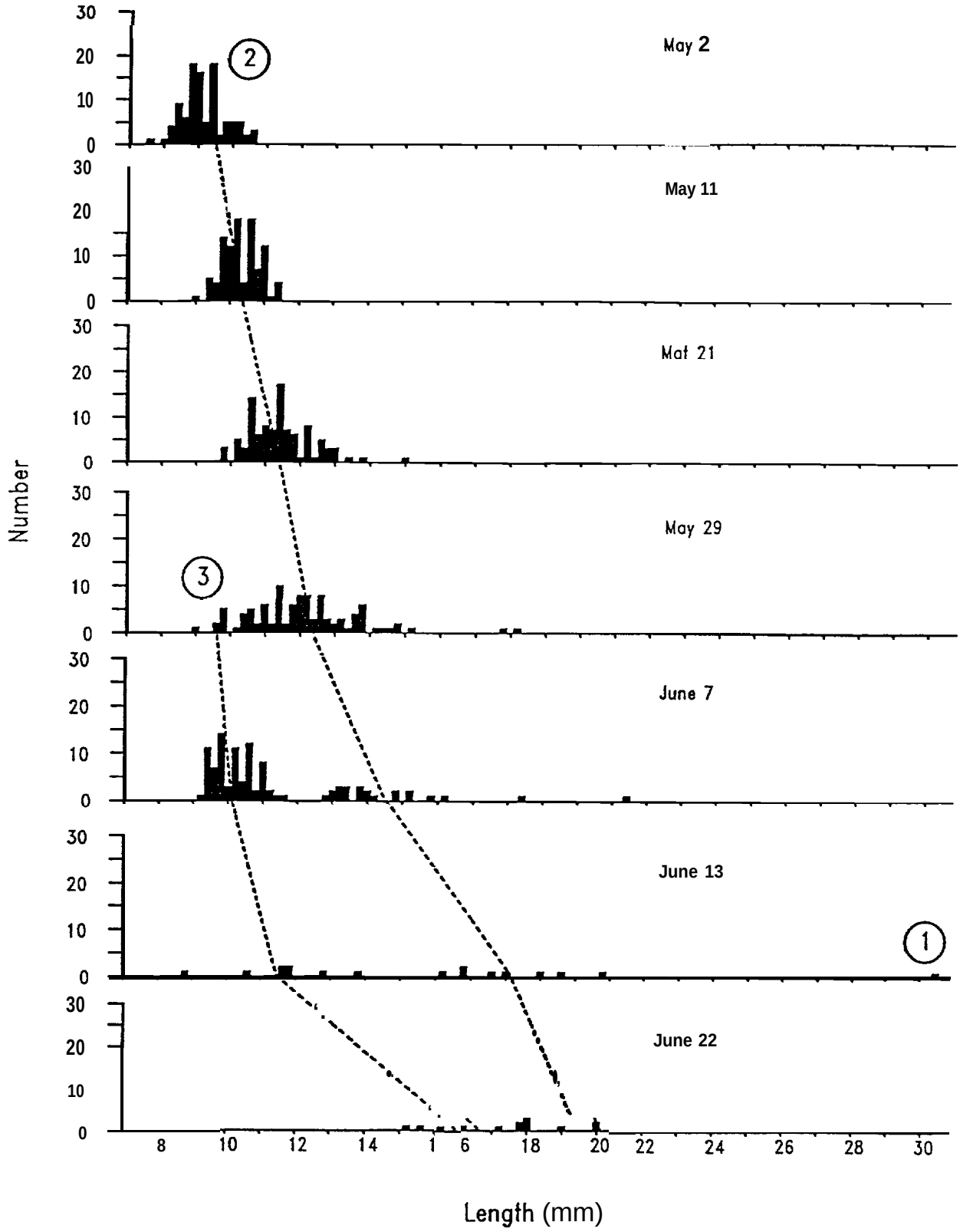


**Figure 5B.** **Length frequency distributions at date for the 3 cohorts of herring larvae found at Fairmount Island. Each cohort is identified by a circled number and by a broken line connecting mean lengths at date.**

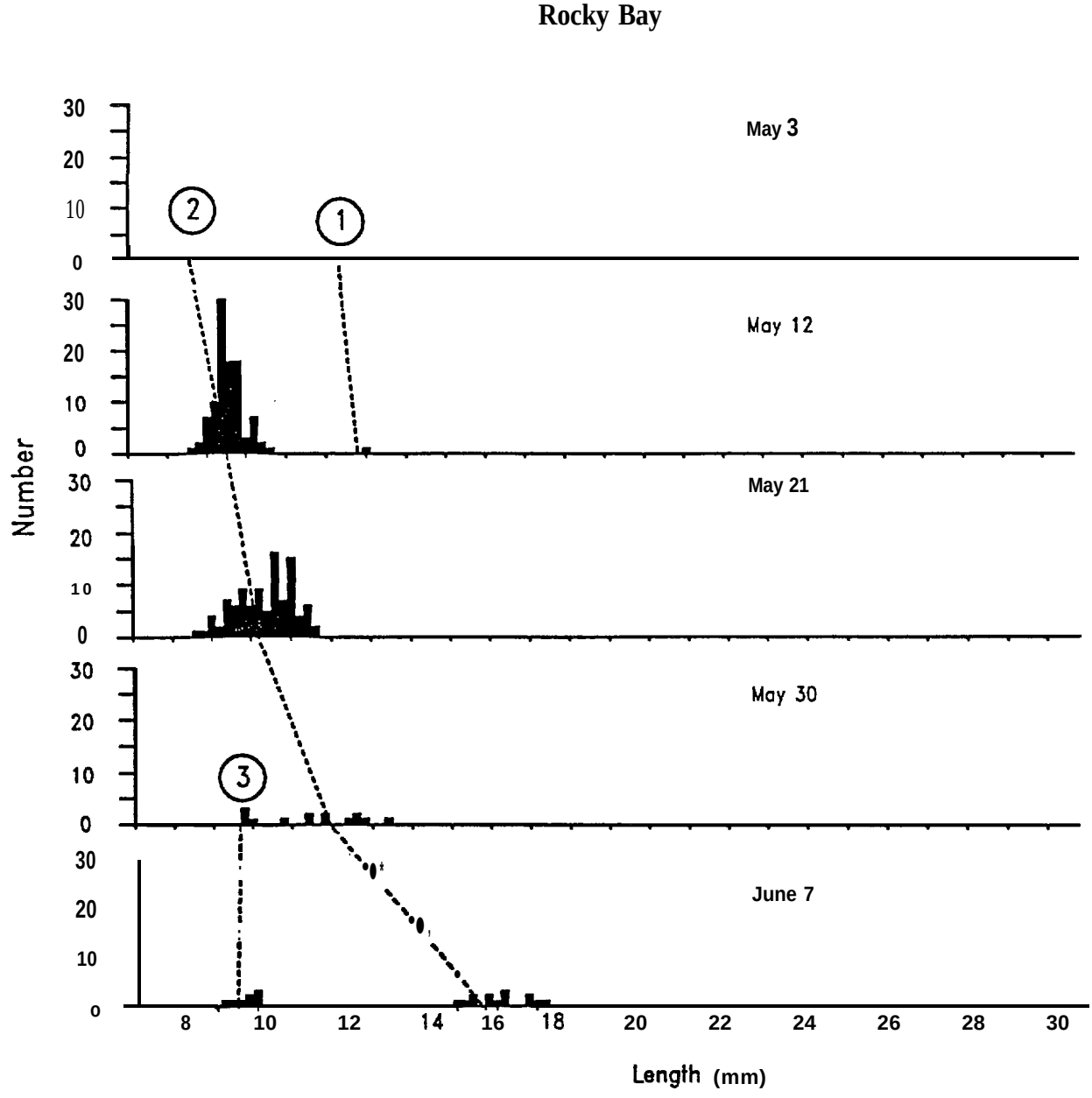


Figure SC. Length frequency distributions at date for the 3 cohorts of herring larvae found at Rocky Bay. Each cohort is identified by a circled number and by a broken I ine connecting mean lengths at date.

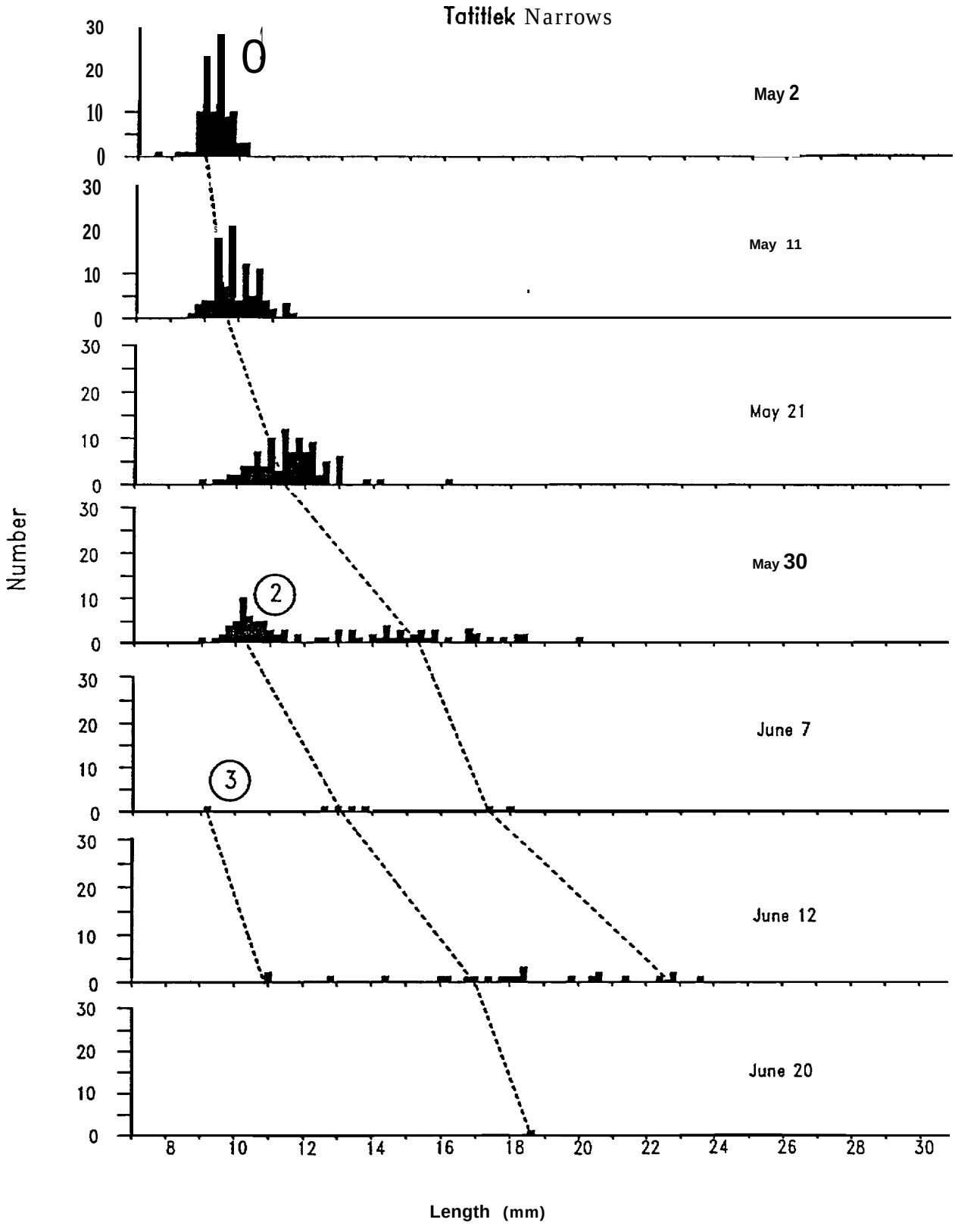


**Figure SD.** **Length frequency distributions at date for the 3 cohorts of herring larvae found at Tatitlek Narrows. Each cohort is identified by a circled number and by a broken line connecting mean lengths at date.**

**station must have been less than 30 km/50 d or 0.6 $km \cdot d^{-1}$ because the shortest straight-line distance between any two of the four stations was at least 30 km and they were sampled over a 50 d period. We have no direct estimates of transport rate (see section 4.8 for a discussion of this topic), but Fig. 15 shows that waves of newly-hatched larvae passed through the sampling stations 1-5 d after hatch. Since the stations were 1-5 km from the egg beds, the rate of transport was within the range 0.2-1.0 $km \cdot d^{-1}$, which supports our assumption that the cohorts did not substantially mix during the May-June period.**

### *4.4 Age* and Growth

#### Gompertz Growth Curves

**Three independent methods were used to estimate the mean dates of hatching of these cohorts and so estimate the age of each larva. The first method was to back-calculate the date of hatching from a modified Gompertz growth curve fit to the lengths at date:**

$$(7)\quad L_D = L_0 \exp\left[\frac{A_0}{a}\{1 - \exp[-a(D - D_0)]\}\right]$$

**where $L_D$ = length (mm) at Julian date D, $L_0$ = length (mm) at hatch, AO = rate of growth ($mm \cdot d^{-1}$) at hatch, a = rate of change ($mm \cdot d^{-1}$) of& and $D_0$ = Julian date of hatch. This growth model differs from the conventional Gompertz curve in the inclusion of Do and the fixing of $L_0$ at 8.8 mm. This was necessary because the age of larvae was known with less certainty than their length at hatching.**

**An estimate of $L_0$ was obtained by regressing mean length against the fraction of yolk sac larvae for all samples that contained yolk sac larvae. Fig. 6 shows that this regression was significant ($P < 0.01$) and that it predicted a mean length of 8.8 mm at 100% yolk sac.**

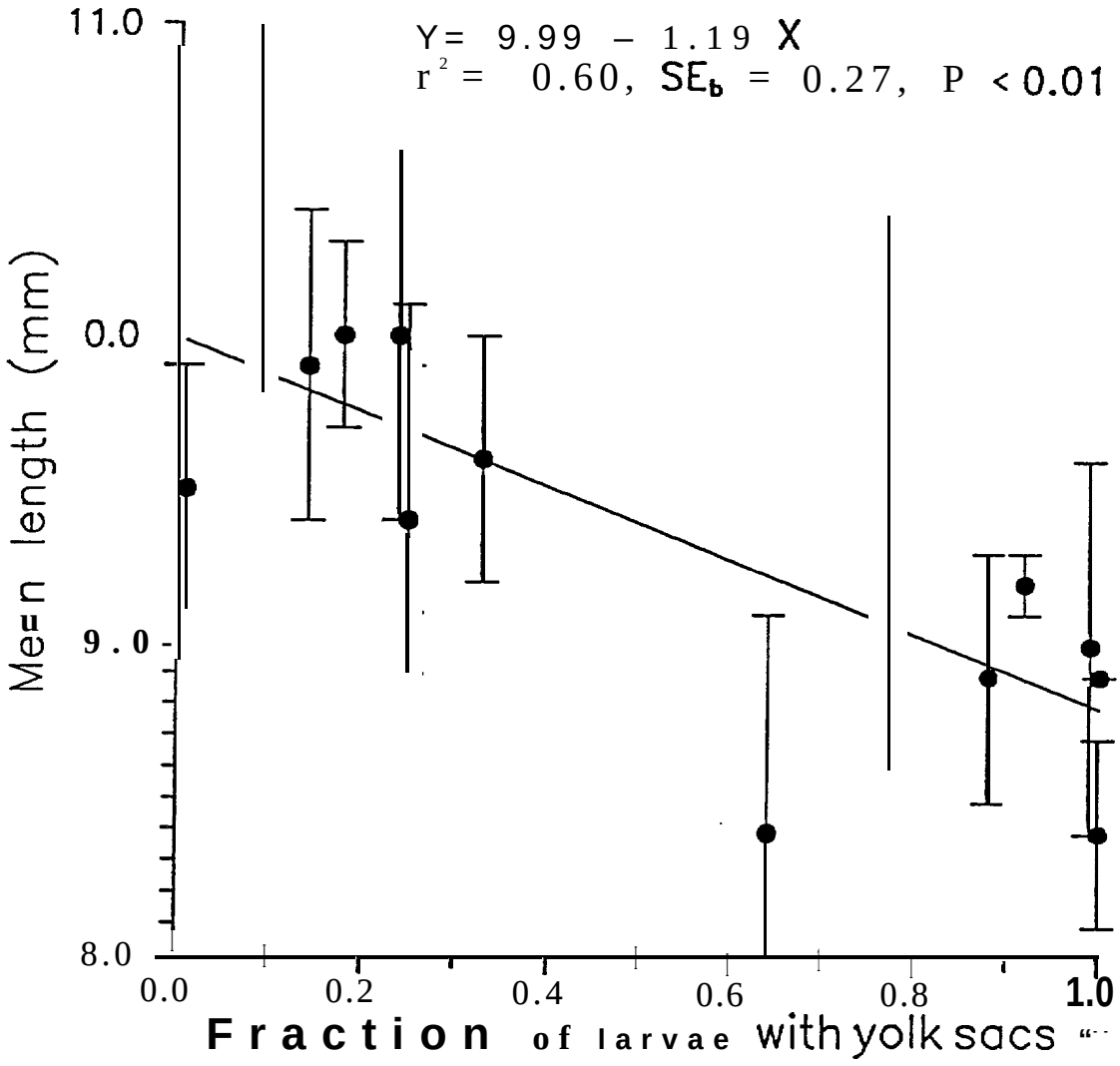


**Figure 6.** **Regression of mean length of herring larvae on the fraction of larvae with yolk sacs. Vertical bars indicate 1 SD. The predicted length at 100% yolk sac, 8.8 mm, is the mean length at hatch.**

The Gompertz models were fit by non-linear regression; parameters are reported in Table 2 only for those cohorts with sufficient sample size for a significant ($P < 0.05$) regression

Duration of Yolk Sac Stage

The second method of estimating hatch dates was based on the fact that the number of days from hatching to exhaustion of yolk in Pacific herring larvae decreases with temperature. Alderdice and Velsen (1971) reported times to yolk exhaustion for 12 combinations of salinity and temperature. Response surface analysis showed that the times were not significantly related to salinity, and that the best relationship with temperature was

$$(8)\quad Y = 40.9T^{-0.84}$$

where Y = time from hatching to yolk exhaustion (d) and T = temperature ($^{o}C$) (McGurk 1989c). Therefore, the average age of a sample containing at least one yolk sac larvae is

$$(9)\quad t = 40.9T^{-0.84}(1-f)$$

where t = age (d) of sample, T = mean temperature of the upper 30 m measured on the same date as the sample was collected, and f = fraction of yolk sac larvae in the sample. The average hatch date of a cohort is the mean of the Julian dates back-calculated from the mean ages weighted by the density of herring larvae measured on that date, i.e.

$$(10)\quad D_0 = [\sum_{i} N_i(D_i - t_i)]/(\sum_{i} N_i)$$

where $N_i$ = density ($m^{-3}$) of herring larvae of sample i collected on Julian date Di. The dates of hatching calculated by this method are shown in Table 3.

**Table 2.**

**Parameters of Gompertz growth models for cohorts of herring larvae. $D_0$ is the Julian date of hatch. Brackets enclose one standard error of the parameters.**

| Site | Cohort | $A_0$ ($mm \cdot d^{-1}$) | a ($mm \cdot d^{-1}$) | $D_0$ | $r^2$ | n |
|---|---|---|---|---|---|---|
| Bass Harbor | 2 | 0.01341 (0.00078) | -0.01491 (0.00321 | 127.1 (0.5) | 0.80 | 362 |
| Fairmount Island | 2 | 0.00815 (0.00092) | -0.02234 (0.00273) | 120.0 (1.4) | 0.78 | 429 |
| Fairmount Island | 3 | 0.00233 (0.00515) | -0.10090 (0.02680) | 140.0 (16.8) | 0.84 | 101 |
| Rocky Bay | 2 | 0.00299 (0.00147) | -0.07560 (0.00752) | 121.7 (4.2) | 0.88 | 227 |
| Tatitlek Narrows | 1 | 0.01129 (0.00103) | -0.03096 (0.00309) | 122.5 (0.9) | 0.87 | 353 |
| Tatitlek Narrows | 2 | 0.05794 (0.01171) | 0.04990 (0.02588) | 147.5 (0.6) | 0.88 | 68 |

**Table 3.**

**Julian dates of hatch estimated from the fraction of yolk sac larvae and the mean water temperature.**

| Site | Cohort | Julian date of capture | Mean T ($^{o}C$) | fraction yolk sac | Age at (d) | Julian date at hatch | Mean $N_i$ ($m^{-3}$) |
|---|---|---|---|---|---|---|---|
| Bass Harbor | 2 | 123 | 5.2 | 0.64 | 3.7 | 119 | 1.307 |
| | | 132 | 5.5 | 0.77 | 2.2 | 130 | 252.844 |
| | | 141 | 6.7 | 0.09 | 7.5 | 133 | 1.878 |
| | | | | | mean | 130 | |
| Fairmount Island | 2 | 122 | 5.4 | 0.88 | 1.2 | 121 | 11.961 |
| | | 132 | 5.5 | 0.24 | 7.4 | 125 | 737.344 |
| | | | | | mean | 125 | |
| Fairmount Island | 3 | 149 | 7.0 | 0.01 | 7.9 | 141 | 0.364 |
| | | 158 | 7.7 | 0.14 | 6.3 | 152 | 2.017 |
| | | | | | mean | 150 | |
| Rocky Bay | 2 | 123 | 5.0 | 1.00 | 0.0 | 123 | 0.015 |
| | | 132 | 5.9 | 0.92 | 0.7 | 131 | 275.270 |
| | | 141 | 6.4 | 0.18 | 7.1 | 134 | 2.861 |
| | | | | | mean | 131 | |
| Rocky Bay | 3 | 150 | 5.7 | 0.25 | 7.1 | 143 | 0.013 |
| | | 158 | 7.9 | 0.25 | 5.4 | 153 | 0.125 |
| | | | | | mean | 152 | |
| Tatitlek Narrows | 1 | 122 | 5.1 | 0.99 | 0.1 | 122 | 152.788 |
| | | 131 | 6.1 | 0.33 | 6.0 | 125 | 237.799 |
| | | | | | mean | 124 | |
| Tatitlek Narrows | 3 | 158 | 8.2 | 1.00 | 0.0 | 158 | |

## Date of Zero Otolith Rings

The third method of back-calculating hatch dates was based on the number of rings in the sagittal otoliths. The first step was to calculate for each cohort the Julian date on which the mean number of rings was zero. These dates were calculated from equation (12) of section 4.5. Since the first visible otolith ring is deposited after the exhaustion of the yolk (McGurk 1984a, Campana et al. 1987), the second step was to calculate the duration of the yolk sac stage from equation (9) and then subtract it from the date of zero ring number. These calculations are summarized in Table 4.

Examination of the hatch dates calculated from the three methods showed that the growth curve method tended to give low estimates, the yolk sac method gave consistently high estimates, and the estimates provided by the otolith method fell in between (Table 5). Since the three methods are independent of each other, their mean was chosen as the best estimate of hatch date. Based on these mean dates, the interval of time between cohorts ranged from 11 to 22 d, and decreased with Julian date and increasing water temperature. The mean interval was 18 (SD = 5, n = 4) d.

## Growth from Mean Lengths at Age

Growth in length of cohorts were calculated from mean lengths at age, i.e.

$$(11)\quad G = (L2 - L_1)/(t_2 - t_1)$$

where G = growth rate ($mm \cdot d^{-1}$) and $L_1$ and L2 are mean lengths at two successive ages $t_1$ and t2, respectively. Growth rates rose from approximately 0.1 $mm \cdot d^{-1}$ in early and mid-May to 0.4 $mm \cdot d^{-1}$ in June (Table 6). The increase was caused by an increase in mean water temperature over the May-June period. Fig. 7 plots the growth rates from Table 6 against the mean water temperature between dates. All negative values of G and one value greater than 1.0 mm d-l were excluded from this plot. A regression of lnG on In(temperature) was highly significant and explained 1% more variance than a regression of lnG on temperature. Covariance analysis indicated that there were no significant (P> 0.05) differences between sites in the growth-temperature relationship.

Table 4.

Julian dates of hatch calculated from the dates of zero rings in the sagittal otoliths and the duration of the yolk sac stage.

| Site | Cohort | Julian date of zero ring no. | Mean T (°C) | Duration of yolk sac stage (d) | Julian date of hatch |
|---|---|---|---|---|---|
| Bass Harbor | 2 | 132.0 | 5.5 | 9.8 | 122.2 |
| Fairmount Island | 2 | 134.8 | 5.5 | 9.7 | 125.1 |
| | 3 | 152.6 | 7.7 | 7.3 | 145.3 |
| Rocky Bay | 2 | 138.2 | 6.1 | 8.9 | 129.3 |
| | 3 | 152.6 | 6.8 | 8.1 | 144.5 |
| Tatitlek Narrows | 1 | 131.2 | 6.7 | 8.2 | 123.0 |
| | 2 | 141.5 | 7.6 | 7.4 | 134.1 |
| | 3 | 152.6 | 8.0 | 7.1 | 145.5 |

Table 5.

Mean Julian dates of hatch estimated from three different methods.

| Cohort | Ageing method | Bass Harbor | Fairmount Island | Rocky Bay | Tatitlek Narrows |
|---|---|---|---|---|---|
| 1 | growth curve | · | - | | 122 |
| | fraction yolk sac | · | - | - | 124 |
| | otolith ring no. | · | | - | 123 |
| | mean | | | - | 123 |
| 2 | growth curve | 127 | 120 | 122 | 147 |
| | fraction yolk sac | 130 | 125 | 131 | · |
| | otolith ring no. | 122 | 125 | 129 | 134 |
| | mean | 126 | 123 | 127 | 140 |
| 3 | growth curve | · | 140 | - | |
| | fraction yolk sac | | 150 | 152 | 158 |
| | otolith ring no. | | 145 | 144 | 145 |
| | mean | | 145 | 148 | 151 |
| | cohort interval (d) | | 22 | 21 | 11-17 |

Notes:

1. Dashes indicate no data available.

Table 6. Population growth in length.

| Date | Site | Mean temp (oC) | Cohort 1 age (d) | Cohort 1 length (mm) mean | Cohort 1 length (mm) SD | Cohort 1 length (mm) n | Cohort 1 growth rate (mmd^-1) | Cohort 2 age (d) | Cohort 2 length (mm) mean | Cohort 2 length (mm) SD | Cohort 2 length (mm) n | Cohort 2 growth rate (mmd^-1) | Cohort 3 age (d) | Cohort 3 length (mm) mean | Cohort 3 length (mm) SD | Cohort 3 length (mm) n | Cohort 3 growth rate (mmd^-1) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-May-89 | Bass Harbour | 5.2 | - | - | - | - | - | -3 | 8.4 | 0.5 | 100 | - | | | - | - | - |
| 12-May-89 | Bass Harbour | 5.5 | - | - | | - | | 6 | 9.5 | 0.5 | 100 | 0.12 | | - | - | - | - |
| 21-May-89 | Bass Harbour | 6.7 | - | - | - | - | - | 15 | 10.4 | 0.9 | 100 | 0.10 | | - | - | - | - |
| 29-May-89 | Bass Harbour | 7.0 | - | - | - | - | - | 23 | 12.9 | 0.8 | 58 | 0.31 | -2 | - | - | - | - |
| 8-Jun-89 | Bass Harbour | 6.7 | - | - | | - | - | 33 | 11.7 | - | 1 | -0.12 | 8 | - | - | - | - |
| 13-Jun-89 | Bass Harbour | 7.9 | - | - | - | - | | 38 | 20.3 | 3.4 | 2 | 1.72 | 13 | 11.6 | 1.8 | 2 | - |
| 21-Jun-89 | Bass Harbour | 9.2 | - | - | - | | - | 46 | 16.9 | - | 1 | -0.43 | 21 | 13.3 | 0.2 | 2 | 0.21 |
| 2-May-89 | Fairmount Island | 5.4 | | - | - | - | - | -1 | 8.9 | 0.6 | 100 | | | - | - | - | - |
| 12-May-89 | Fairmount Island | 5.5 | | - | - | | - | 9 | 10.0 | 0.5 | 100 | 0.11 | | | - | - | - |
| 21-May-89 | Fairmount Island | 6.1 | | | | - | | 18 | 11.1 | 0.9 | 100 | 0.12 | -4 | - | - | - | |
| 29-May-89 | Fairmount Island | 7.0 | | | - | - | - | 26 | 12.0 | 1.4 | 90 | 0.11 | 4 | 9.5 | 0.4 | 10 | |
| 7-Jun-89 | Fairmount Island | 7.7 | | - | - | - | - | 35 | 14.2 | 2.0 | 23 | 0.24 | 13 | 9.9 | 0.6 | 77 | 0.04 |
| 13-Jun-89 | Fairmount Island | 7.5 | | 30.0 | - | 1 | - | 41 | 17.8 | 1.6 | 8 | 0.60 | 19 | 11.3 | 1.5 | 8 | 0.23 |
| 22-Jun-89 | Fairmount Island | 8.6 | | - | | | - | 50 | 19.8 | 2.8 | 8 | 0.22 | 28 | 16.4 | 1.1 | 6 | 0.57 |
| 3-May-89 | Rocky Bay | 5.0 | | 12.1 | - | 1 | | -4 | 8.4 | 0.3 | 3 | - | | - | - | - | |
| 12-May-89 | Rocky Bay | 5.9 | | 12.6 | - | 1 | 0.06 | 5 | 9.2 | 0.4 | 99 | 0.09 | | - | - | - | |
| 21-May-89 | Rocky Bay | 6.4 | | | - | | | 14 | 10.0 | 0.7 | 100 | 0.09 | -9 | - | - | - | |
| 30-May-89 | Rocky Bay | 5.7 | | - | | | | 23 | 11.8 | 0.8 | 10 | 0.20 | 2 | "9.6 | 0.1 | 4 | |
| 7-Jun-89 | Rocky Bay | 7.9 | | - | | | | 31 | 15.7 | 0.8 | 15 | 0.49 | 10 | 9.4 | 0.3 | 8 | -0.02 |
| 2-May-89 | Tatitlek Narrows | 5.1 | -1 | 9.0 | 0.4 | 100 | | | | | | | | - | - | - | |

Table 6. Population growth in length. (Continued)

| Date | Site | Mean temp (oC) | Cohort 1 age (d) | Cohort 1 length (mm) mean | Cohort 1 length (mm) SD | Cohort 1 length (mm) n | Cohort 1 growth rate (mmd^-1) | Cohort 2 age (d) | Cohort 2 length (mm) mean | Cohort 2 length (mm) SD | Cohort 2 length (mm) n | Cohort 2 growth rate (mmd^-1) | Cohort 3 age (d) | Cohort 3 length (mm) mean | Cohort 3 length (mm) SD | Cohort 3 length (mm) n | Cohort 3 growth rate (mmd^-1) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11-May-89 | Tatitlek Narrows | 6.1 | 8 | 9.6 | 0.6 | 100 | 0 | 0 | 7 | - | - | - | - | - | - | - | - |
| 20-May-89 | Tatitlek Narrows | 7.3 | 17 | 11.2 | 1.0 | 100 | 0 | 1 | 8 | 0 | - | - | - | - | - | - | - |
| 30-May-89 | Tatitlek Narrows | 7.8 | 27 | 15.1 | 1.8 | 43 | 0.39 | 10 | 10.1 | 0.6 | 49 | - | -1 | - | - | - | - |
| 7-Jun-89 | Tatitlek Narrows | 8.2 | 35 | 17.4 | 0.4 | 2 | 0.29 | 18 | 12.9 | 0.6 | 4 | 0.35 | 7 | 8.9 | - | 1 | - |
| 12-Jun-89 | Tatitlek Narrows | 8.5 | 40 | 21.6 | 1.2 | 8 | 0.84 | 23 | 16.8 | 1.8 | 14 | 0.78 | 12 | 10.7 | 0.0 | 2 | 0.36 |
| 20-Jun-89 | Tatitlek Narrows | 8 | 7 | 4 | 8 | - | - | - | - | 31 | 18.3 | - | 1 | 0.19 | 20 | - | - | - | - |

Notes:

1. growth rate = (L2 - L1)/(t2 - t1).

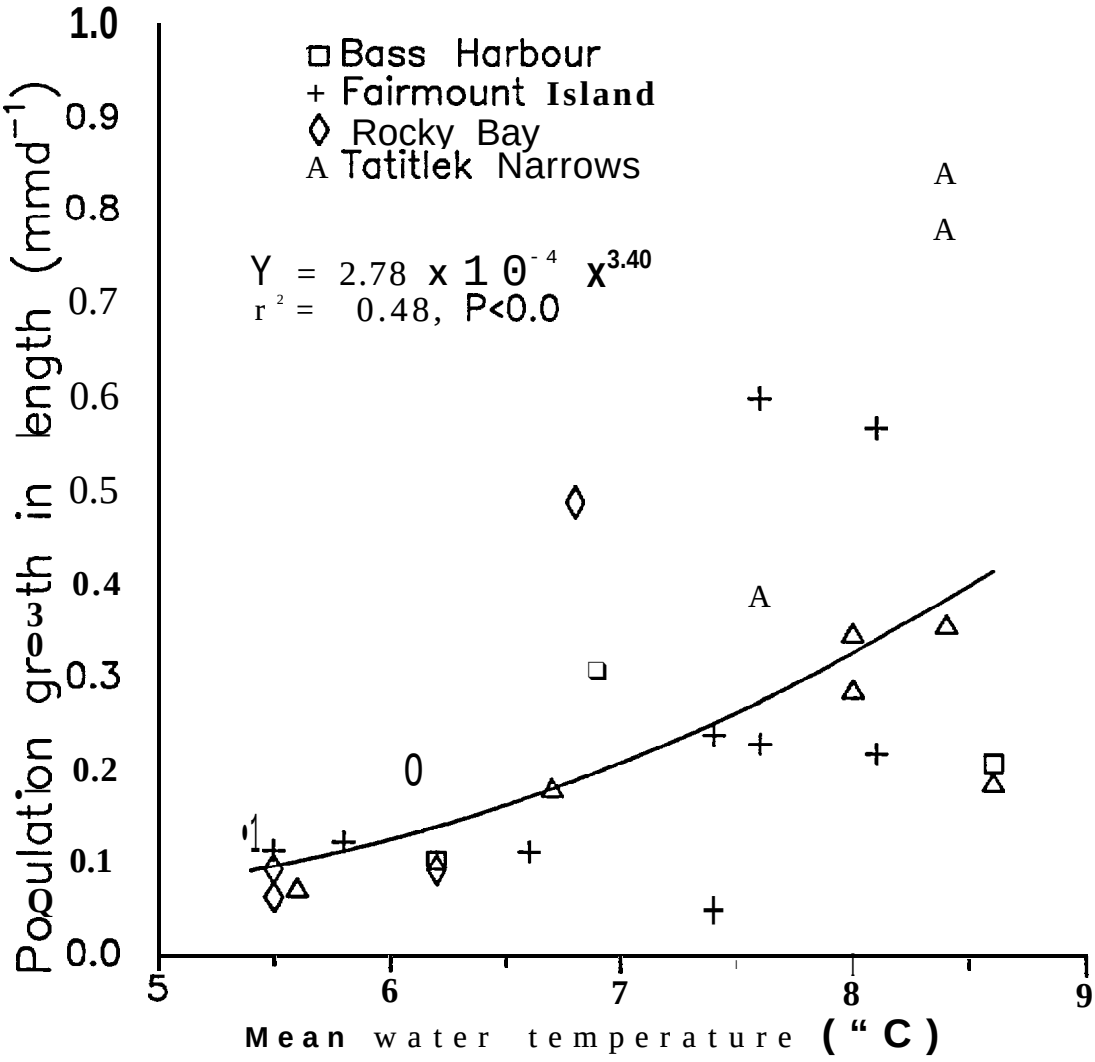


**Figure 7.** **Regression of population growth rates on mean water temperature for the four sites. No differences in growth rate were found between sites.**

## *4.5* Otolith Analysis

### Fish Length-Otolith Radius Relationship

Recent growth rates of individual larvae were calculated from the average width of the outer five rings of the sagittal otolith. The first step in converting ring width to growth rate was to calculate a regression between fish length and otolith radius. Radius was first linearized by ln-transformation. Then multiple regression with dummy variables was used to determine if either the intercept or slope of the relationship was significantly different between cohorts or sites. Analysis on a cohort basis was first attempted, but failed because there were not enough larvae to give the degrees of freedom necessary to allow the inclusion of one dummy variable for each of the eight cohorts plus an interaction of the dummy variable and ln(radius). Therefore, the analysis was restricted to a site basis with one dummy variable and one interaction term for each of the four sites. The final regression model was:

$$(12)\quad L = -4.9621 + 6.2217 \ln R + 0.9337 g_4$$
$$(SE)\quad (0.9526) \qquad (0.3115) \qquad (0.3354)$$
$$r^2 = 0.84,\ n = 84,\ P < 0.001$$

where L = fish length (mm), R = radius ($\mu$m) of otolith, and $g_4$ = dummy variable with a value of 1 for Tatitlek Narrows fish and O for the other three sites.

The model shows that the rate of increase of otolith radius with fish length was the same in all larvae, but that herring larvae from Tatitlek Narrows had significantly smaller otoliths at any length than larvae from the other three sites (Fig. 8). This finding indicates that herring larvae from Tatitlek Narrows had higher average growth rates than fish from the other three sites. Reznick et al. (1989) and Secor and Dean (1989) recently reported that fast growing guppies, Poecilia reticulata, and striped bass, Morone saxitilis, respectively, have smaller otoliths than slower growing fish of the same size.

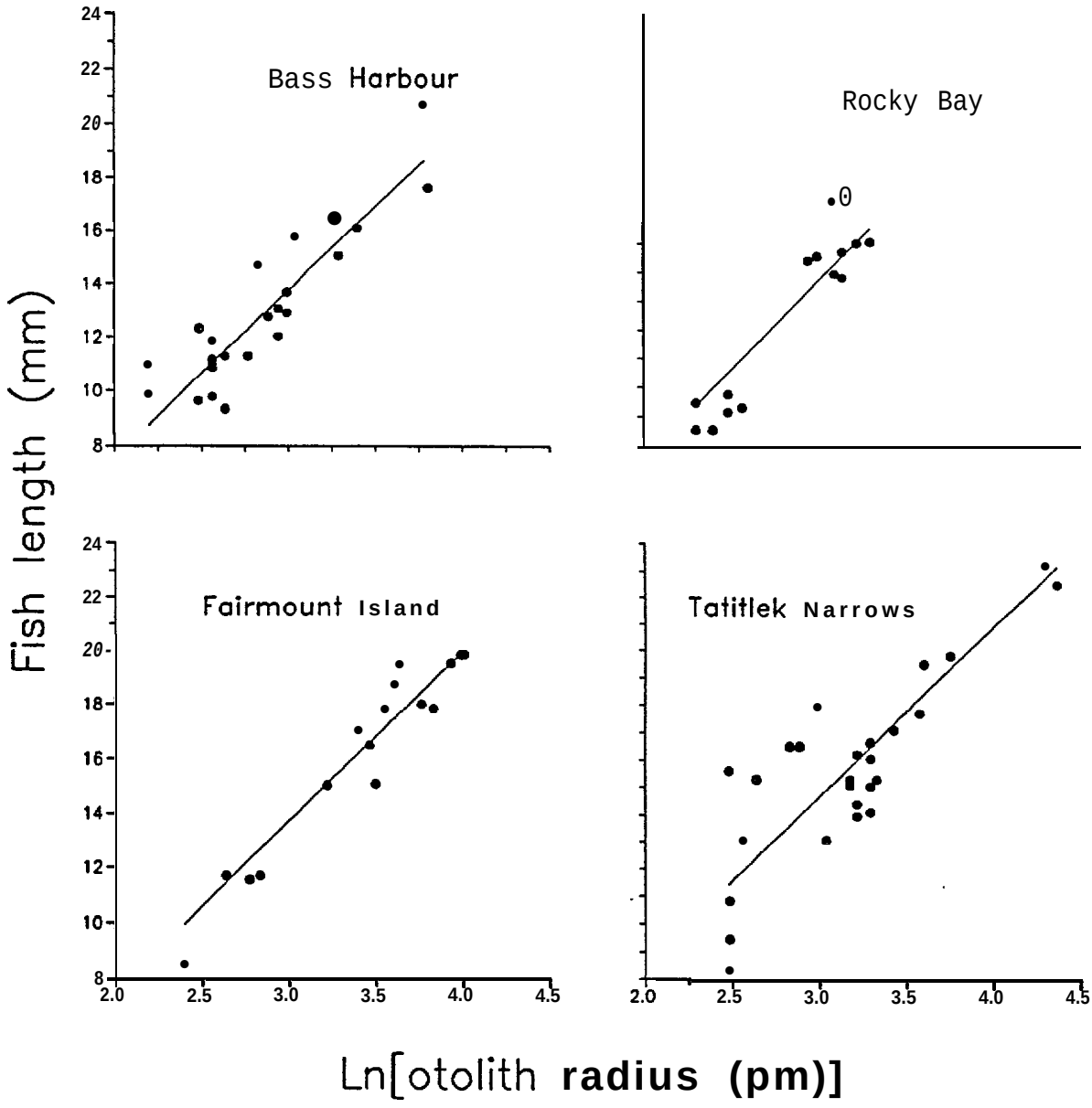


**Figure 8.** **Regression of fish length on ln(otolith radius) for herring larvae from four sites. See text and equation (12) for detail. Larvae from Tatitlek Narrows had significantly smaller otoliths at any length than larvae from other sites.**

Equation (12) was used to convert an increment in otolith radius, in this case the average width of one otolith ring, to an increment in fish length, i.e.

(13) $L2 - L_1 = 6.2217\ln(R_2/R_1)$

where $L_1$ = length (mm) before deposition of a ring, L2 = length (mm) after deposition of that ring, $R_1$ = otolith radius ($\mu$m) before deposition, and R2 = otolith radius ($\mu$m) after deposition. This increment in length was converted to an increment in dry weight using a weight-length regression described in section 4.6.

Otolith Ring Deposition Rate

The second step in converting ring width to growth rate was to estimate the average time required to deposit one ring. This was done with a covariance analysis of the ring number-Julian date relationship. One dummy variable and its corresponding interaction term was inserted for each of the eight cohorts that had otolith ring number data. Each dummy variable had a value of 1 for its cohort and O for all other cohorts. The dummy variables were

| Site | Cohort | Variable |
|---|---|---|
| Bass Harbor | 2 | $x_1$ |
| Fairmount Island | 2 | $x_2$ |
| Fairmount Island | 3 | x3 |
| Rocky Bay | 2 | $x_4$ |
| Rocky Bay | 3 | x5 |
| Tatitlek Narrows | 1 | $x_6$ |
| Tatitlek Narrows | 2 | x7 |
| Tatitlek Narrows | 3 | X8 |

The multiple regression model that explained the most variance (? = 0.81, n = 84) in the ring data with all significant (P< 0.0001) coefficients was

(14)

| variable | coefficient | SE |
|---|---|---|
| intercept | -142.7 | 12.2 |
| Julian date | 0.9347 | 0.0756 |
| $x_1$ | 19.25 | 2.00 |
| $x_2$ | 16.67 | 2.11 |
| $x_4$ | 13.46 | 2.08 |
| $x_6$ x Julian date | 0.1530 | 0.0124 |
| x7x Julian date | 0.0736 | 0.0170 |

The fit of the model to the data is shown in Fig. 9.

The model shows that the rate of ring deposition was a constant 0.93 rings·$d^{-1}$ for all fish except cohorts 1 and 2 from Tatitlek Narrows; they had significantly higher deposition rates: 1.09 and 1.01 rings·$d^{-1}$, respectively. This result supports the conclusions derived from the analysis of the fish length-otolith radius relationship; growth of herring larvae was faster in fish from Tatitlek Narrows than in fish from the other three sites.

Recent Growth Rates

The third step was to calculate recent growth rates as

(15) $G_w = (1/t_i)\ln(W_2/W_1)$

where $G_w$ = recent growth rate (%·$d^{-1}$), ti = the time (d) required to deposit one ring in cohort i, W2 = dry weight ($\mu$g) of larvae after deposition of one ring and $W_1$ = dry weight ($\mu$g) before deposition Multiple regression analysis was used to test the hypothesis that $G_w$ was significantly different between sites due to a non-environmental, i.e. oil-related, cause. $G_w$ was regressed on age of fish larvae, mean water temperature of the upper 30 m, prey concentration, four dummy variables corresponding to the four sites, and the interactions of these variables. The model that explained the most variance with all significant ($P < 0.001$) coefficients was

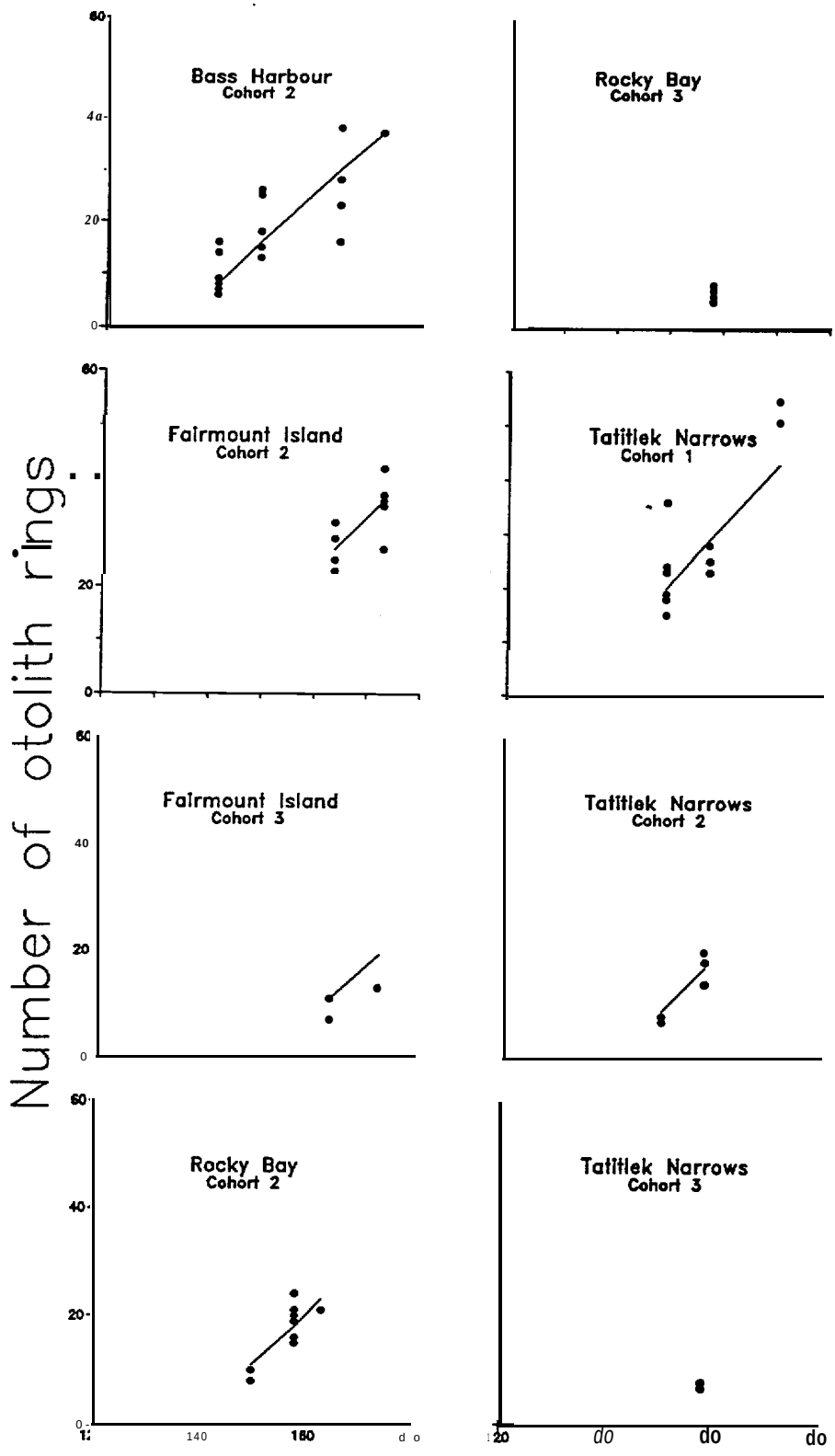


**Figure 9.** **Observed (closed circles) and predicted (lines) number of otolith rings against Julian date for eight cohorts of herring larvae. The rates of ring deposition for cohorts 1 and 2 from Tatitlek Narrows were both significantly higher than the rates for the other six cohorts.**

(16) $G_w = 4.6108 + 0.1281t - 4.4x10^{-5}t^3$
(SE) (0.5802) (0.0320) ($1.1x10^{-5}$)
? = 0.16, n = 82

where t = age (d) of larvae (Fig. 10). This result is not unexpected; dome-shaped relationships between $G_w$ and age of herring larve have previously been reported by Oiestad (1983) and McGurk (1989b). The model shows that there were no significant effects of environmental factors or of site (i.e. oil) on recent growth rate.

## 4.6 Morphometry

### Weight-Length Relationship

A logistic model best described the relationship between dry weight and length of all herring larvae (Fig. 11). The model was fit to the ln-transformed weight and length with non-linear regression.

### Morphometric Condition

The mean CFs for the four sites ranged from 0.357 to 0.995 and none were significantly (P> 0.05) different from each other or from zero. The mean CF for the pooled data was 0.717 (SD= 1.300, n=200), which was also not significantly (P> 0.05) different from zero.

The distribution of larvae with morphometric condition shows that the slope of the right-hand limb of the catch curve was much steeper than the slope of the left-hand limb, indicating a rapid loss of poorly-conditioned fish from the populations (Fig. 12). Presumably, larvae with condition greater than about 4.25 were not available to the towed plankton nets because they were either dead from starvation or predation or because they had lost osmotic control and had fallen out of the water column. The single larva with a CF value of 5.25 may have been an artifact. This feature of Fig. 12 means that the equivalent number of days of starvation can be estimated from the morphometric condition factor by assuming direct proportionality, i.e.

(17) $ns = (ns_{,max}/CF_{max})CF, CF \geq 0$

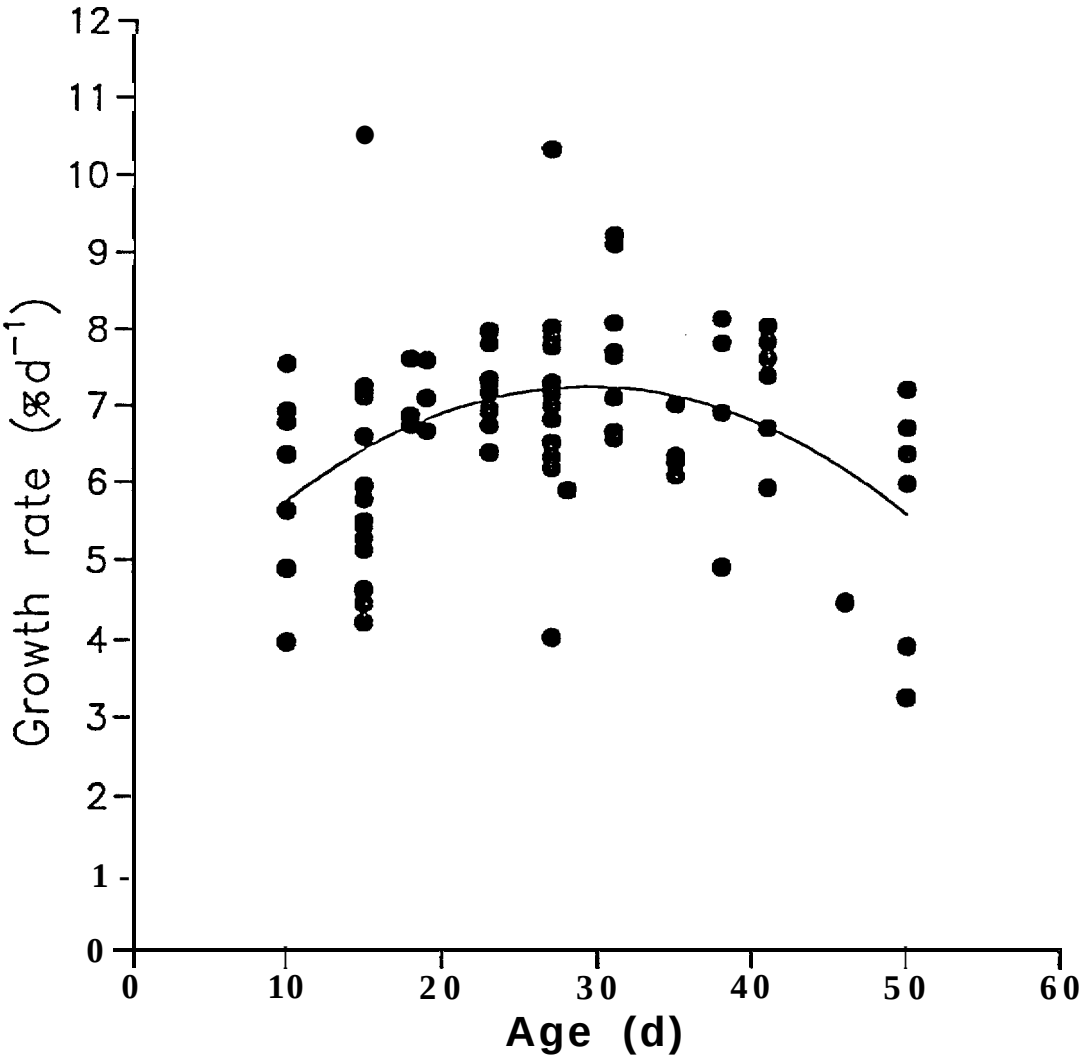


**Figure 10.** **Regression of recent growth rate on age for all herring larvae. See text and equation (16).**

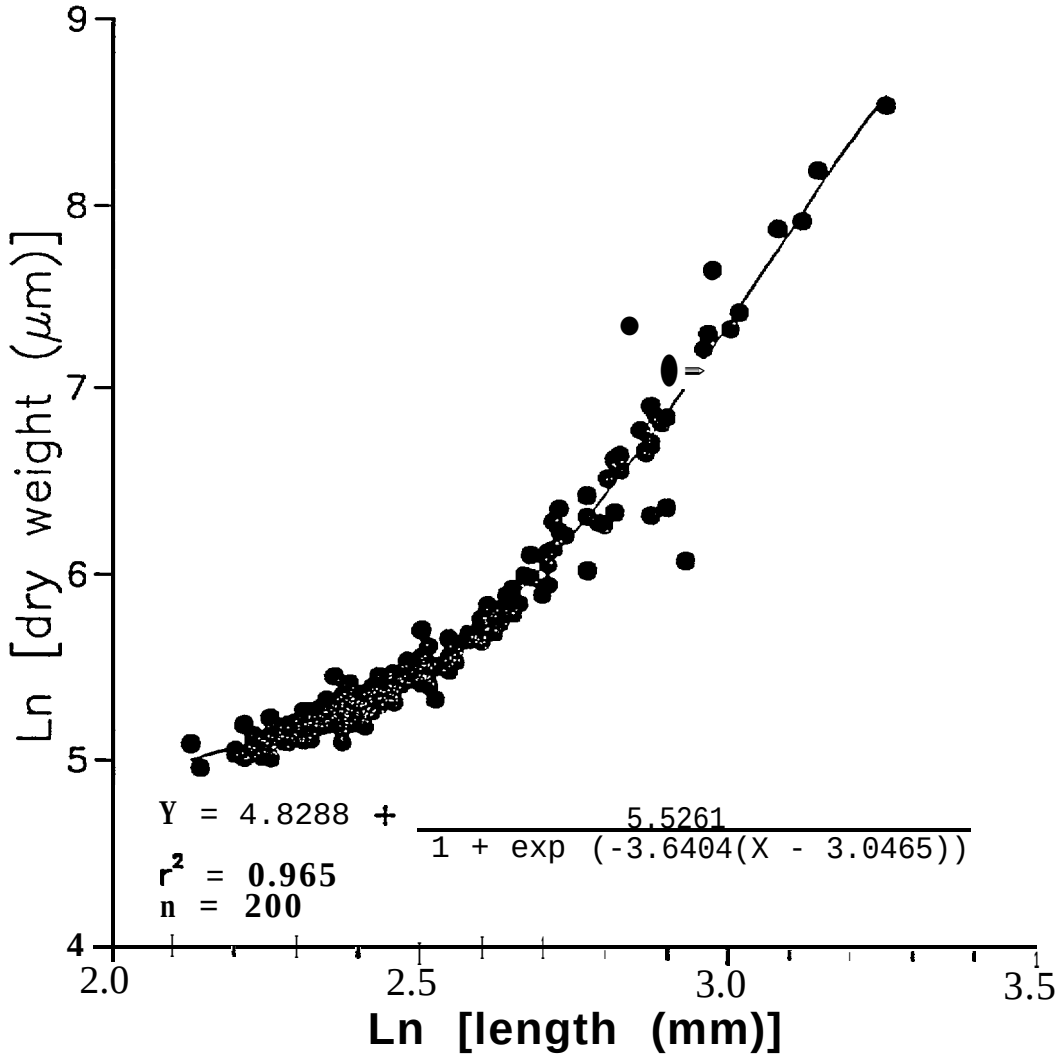


**Figure 11.** Logistic regression of In(dry weight) on ln(length) for all herring larvae.

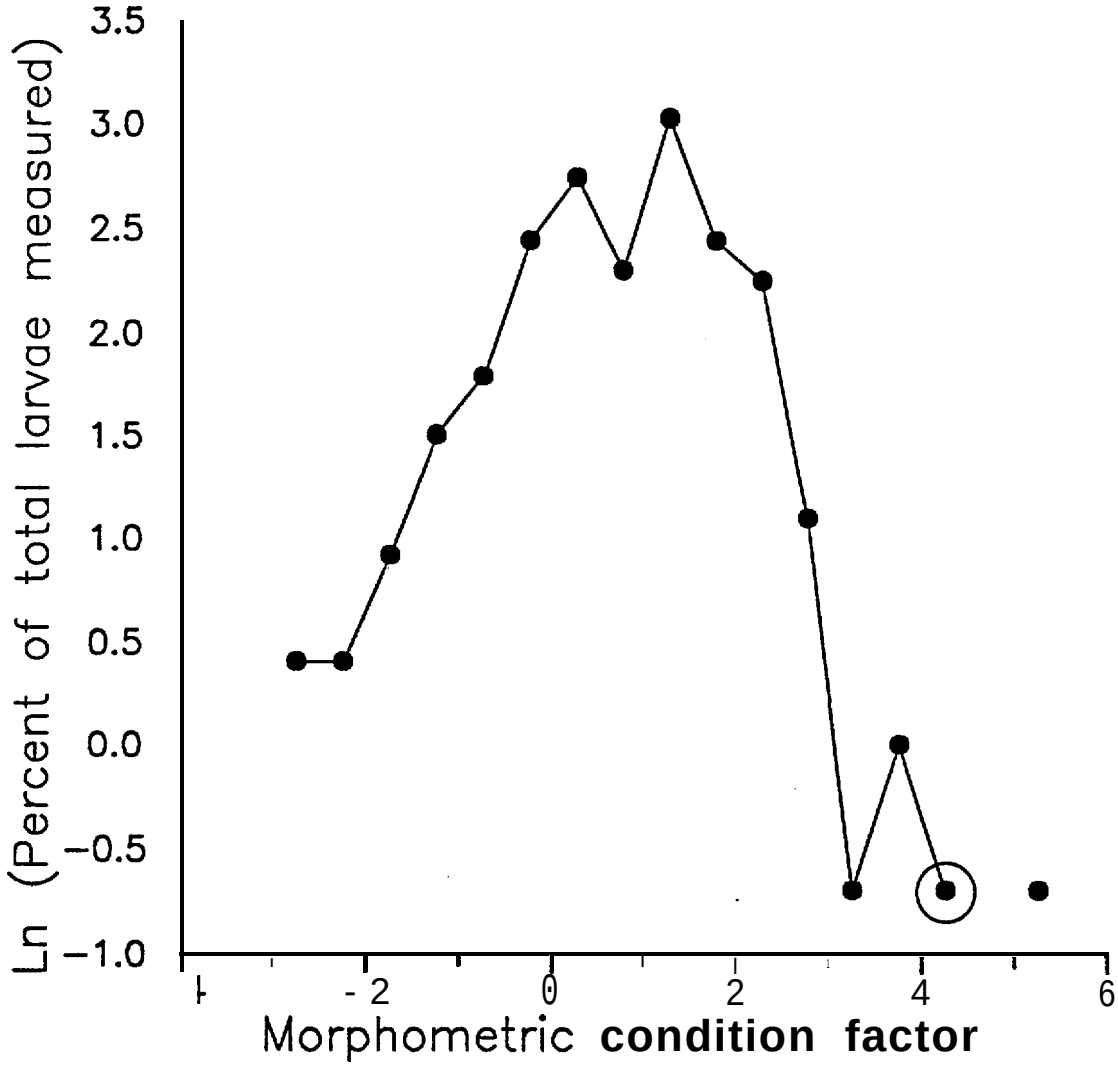


**Figure 12.** **Frequency distribution of morphometric condition of herring larvae. The mean CF was 0.717 and the poorest conditioned larvae (circled) had a CF of** 4.25.

where $n_s$ = duration (d) of period of starvation, $n_{s,max}$ = the maximum number of days that a Pacific herring larva can starve before falling out of the water column and so becoming unavailable to plankton nets, and $CF_{max}$ = the largest positive value of CF found in the catches. McGurk (1984b) reported that larvae reared in water temperatures of 6-8°C and starved from hatch begin falling out of the water column at an age of 11.5 d and complete fallout at an age of 15 d. Therefore, the median age of fallout at these temperatures was 13 d. Since larvae reared at these temperatures are capable of feeding at an age of 3 d, $n_{s,max}$ was 10 d. Therefore, the ratio of $n_{s,max}$ to $CF_{max}$ is 2.35 days per unit of positive condition, which means that the mean CF of 0.717 corresponded to 1.7 d of food deprivation, a negligible period of time.

Even though there were no differences between sites in the mean condition of herring larvae, differences may have existed in the age trajectory of condition. Multiple regression of morphometric condition on age, dummy variables for cohom, and the interactions of age and cohort showed that the maximum amount of variance in condition, CF, was explained by the following model

| | | | | |
|---|---|---|---|---|
| (18) | CF = 2.0648- | 0.0784t + | 0.0264x2t + | 0.0584x3t |
| | (SE) (0.1510) | (0.0070) | (0.0067) | (0.0161) |
| | (P) (<0.0001) | (<0.0001) | (0.0001) | (0.0004) |

$r^2$ = 0.38, n = 199

where x2 = 1 for cohort 2 of Fairmount Island and O for all other cohorts, and x3 = 1 for cohort 3 of Fairmount Island and O for all other cohorts. This model indicates that condition increased with age at a significantly slower rate in herring larvae from Fairmount Island than in fish from the other three sites (Fig. 13). Since Fairmount was a control site this effect was not related to oil.

### 4.7 RNA-DNA Ratios

No significant correlations were found between the RNA-DNA ratios of herring larvae and their age and size (length and dry weight), or with environmental variables (site, mean water temperature and mean prey concentration) (Fig. 14). The mean RNA-DNA ratios of Table 7 show that the two control sites have the highest mean ratios, which suggests a subtle effect of oil on instantaneous growth

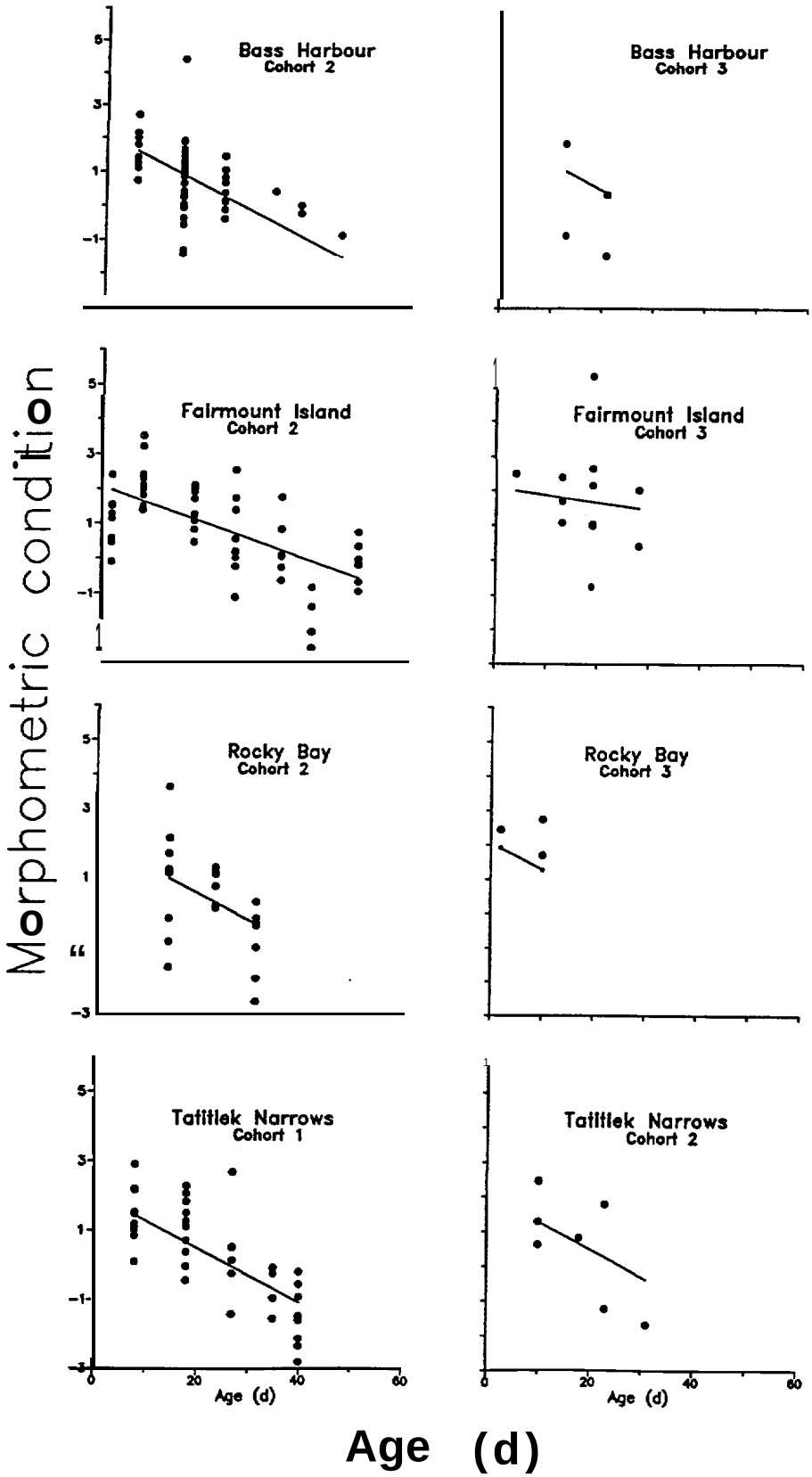


**Figure 13.** **Regression of morphometric condition factor on age for herring larvae from eight cohorts at four sites. See text for equation (18).**

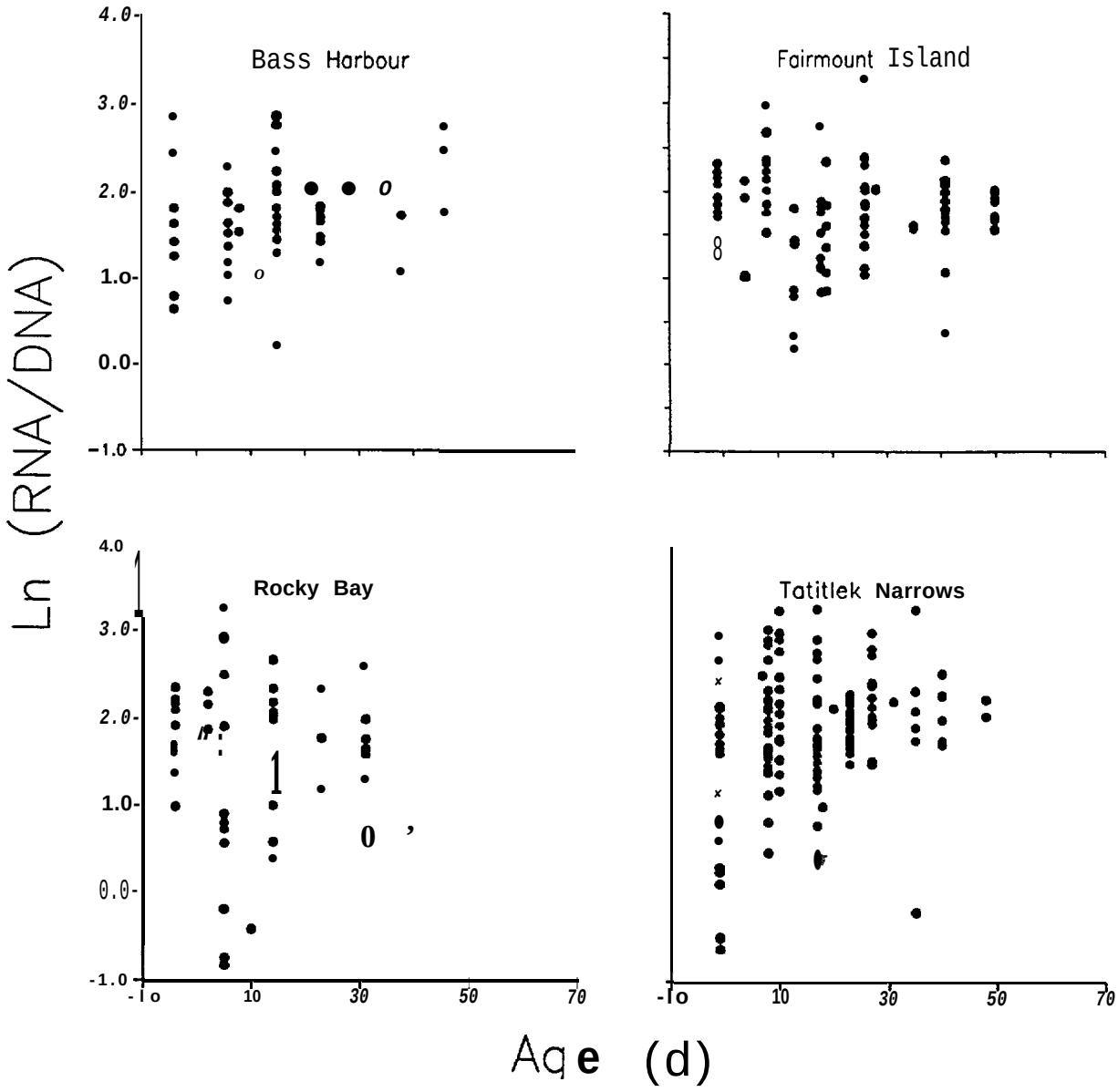


**Figure 14.** **Plots of RNA-DNA ratio of herring larvae against age; showing no change with age and no differences between sites.**

**Table 7.**

**Mean ln(RNA-DNA) and the equivalent geometric mean ratios for the four sites.**

| Site | mean ln(RNA/DNA) | SD | n | geometric mean RNA/DNA |
|---|---|---|---|---|
| Bass Harbor | 1.6849 | 0.5757 | 53 | 5.392 |
| Fairmount Island | 1.7287 | 0.5192 | 96 | 5.633 |
| Rocky Bay | 1.5362 | 0.8258 | 62 | 4.647 |
| Tatitlek Narrows | 1.8545 | 0.6735 | 159 | 6.388 |
| Grand mean | 1.7442 | 0.6604 | 370 | 5.721 |

rates. However, the variance of the ratios is too large to demonstrate this effect. The main reason for the large variance is that all of the larvae from Bass Harbor, Fairmount Island and Rocky Bay and 100 of the 159 larvae from Tatitlek Narrows were analysed with Karsten and Wollenberger's (1972, 1977) method, which is less accurate and less precise than the best method that is now available, that of Clemmessen (1988). This conclusion is a finding of this study and is described in Appendix A. All of the RNA-DNA ratios shown in Fig. 14 were either measured with Clemmessen's (1988) method or were calculated from RNA and DNA concentrations that were corrected for differences in accuracy between the two methods. However, the latter ratios were not corrected for differences in precision because there is no satisfactory method of correcting for precision that would not bias subsequent analysis of the corrected data. It is important to stress that the RNA-DNA ratios shown in Fig. 14 must not be used as absolute RNA-DNA ratios, but only as relative ratios, i.e. their mean is reliable but their range is not biologically reasonable.

The mean RNA-DNA ratios for each of the four sites are smaller than those reported by Fukuda et al. (1986) and Clemmessen (1987), but similar to those reported by Robinson and Ware (1988). We are reluctant to calculate protein growth rates from our RNA-DNA ratios using Buckley's (1984) equation because we are uncertain whether RNA-DNA ratios measured with Clemmessen's (1988) method are comparable to Buckley's (1984) data, which was made using the Schmidt-Thannhauser (1945) method. For the same reason we are also reluctant to use Clemmessen's (1987) equation to calculate the number of degree-days of starvation from RNA-DNA ratios.

### 4.8 Mortality and Transport

Mortality and transport of herring larvae were estimated with two population models incorporating different assumptions about the time trajectories of diffusion and mortality. It was necessary to combine the two processes of mortality and transport in order to correct for the fact that larvae are recruited to and "lost" from a sampling station due to both processes rather than mortality alone (McGurk 1989a), and because it is the best explanation for the ascending limbs of the catch curves shown in Fig. 15.

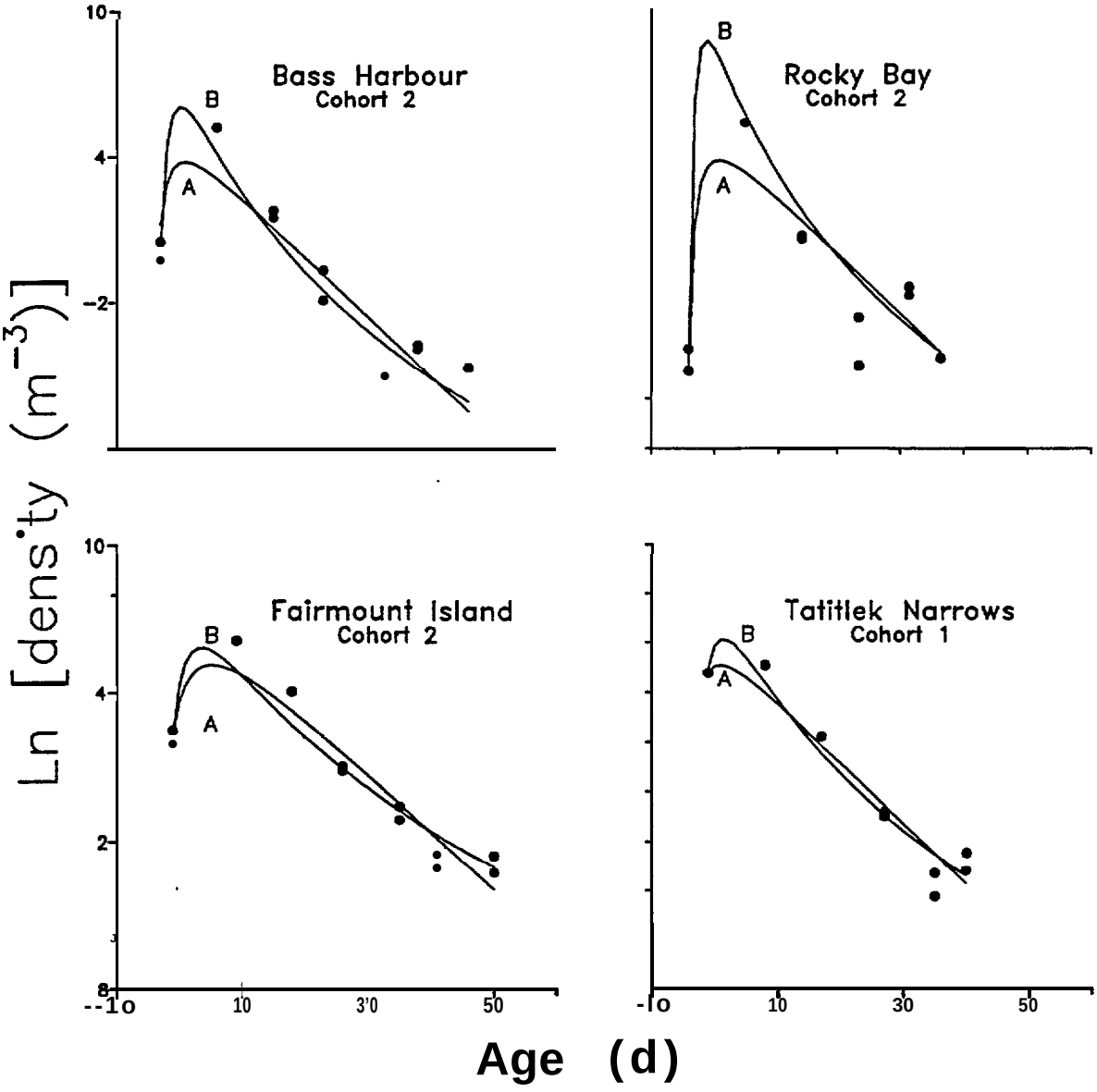


**Figure 15.** **Densities of herring larvae at age and site. Solid lines are densities predicted from diffusion-mortality models. Model A [equation (22)] incorporates Fickian diffusion and constant mortality, and model B [equation (23)] incorporates time-dependent diffusion and mortality.**

The simplest model assumes Fickian diffusion and a constant mortality with age

$$(19)\quad N = \frac{C}{4\pi HKt} \exp\left[\frac{-x^2}{4Kt} - Zt\right]$$

where N = density ($m^{-3}$) of herring larvae at age t (d), C = number of herring larvae hatched at t = O, H = maximum depth (m) of distribution of herring larvae, x = distance (m) between the hatch site and the plankton station, K = coefficient of Fickian diffusion ($m^2 \cdot d^{-1}$), and Z = coefficient of mortality (d-l) (Okubo 1980). This model was fit to the herring larval densities of this study because it was used previously for Pacific herring larvae by McGurk (1989a), and because its parameters are relatively easy to interpret. However, as will be shown below, it does not provide the best fit to the data, probably because its assumptions are unrealistic. For example, Fickian diffusion assumes that horizontal variance is constant with time from release, but Okubo (1971) showed that horizontal variance of dye particles in the sea is actually proportional to time to a power between 2 and 3. Mortality of fish is known to decrease with time over the egg to juvenile stages (e.g. Bailey and Houde 1989) but has usually been expressed as a constant mortality over single stages for reasons of computational convenience. Recent studies have reported that larval mortality declines exponentially with age for at least one species, jack mackerel, Trachurus symmetricus (Hewitt et al. 1985), according to the form $N = N_0 t^{-\beta}$, where $N_0$ = density ($m^{-3}$) at hatch (t = O) and $\beta$ = coefficient of mortality, i.e. $Z = \beta / t$.

The second model was based on two other solutions to the basic equations of tubulent diffusion that assume horizontal variance increases exponentially with time:

Joseph-Sendner:

$$(20a)\quad N = \frac{C}{2\pi HP^2 t^2} \exp\left(\frac{-x}{Pt}\right)$$

Ozmidov:

$$(20b)\quad N = \frac{C}{6\pi H y^3 t^3} \exp\left(-\frac{x^{2/3}}{yt}\right)$$

where P = diffusion velocity ($m \cdot d^{-1}$) and y = energy dissipation parameter ($m^{2/3} \cdot d^{-1}$) (Okubo 1980). Adding a term for a non-constant mortality rate gives

$$(21a)\quad N = \frac{Ct^{(2+\beta)}}{2\pi H P^2} \exp\left(-\frac{x}{Pt}\right)$$

$$(21b)\quad N = \frac{Ct^{(3+\beta)}}{6\pi H y^3} \exp\left(-\frac{x^{2/3}}{yt}\right)$$

Equation (19) was fit to the avoidance-corrected herring densities with non-linear multiple regression after ln-transformation and rearrangement, i.e.

$$(22)\quad \ln(N) = b_0 - \ln(t) - b_1 - Zt$$

where $b_0 = \ln(C/4\pi HK)$ and $b_1 = x^2/4K$. Distance, x, was treated as part of a coefficient rather than a variable because it was not possible to identify a single accurate value of x for each site; the larvae of each of the four major cohorts hatched from beaches which extended for kilometers along the nearby shores. However, the average value of x was probably within the range of 1-5 km for all four sites. Equation (21) was fit using linear multiple regression i.e.

$$(23)\quad \ln(N) = b_0 - b_1 \ln(t) - b_2 t^{-1}$$

where $b0 = C/2\pi HP^2$ or $C/6\pi Hy^3$, $b_1 = 2+\beta$ or $3+\beta$, and b2 = x/P or $x^{2/3}/y$. The uncertainty about whether equation (21a) is more or less correct than equation (21b) is the reason why the second type of population model is less easy to interpret than equation (19).

Only non-zero densities were used because zero counts could not be assigned an age, and because they do not represent true zero densities but only indicate that the density of the larvae was lower than the limit of detection of the gear.

In order to identify statistically significant differences in mortality and transport coefficients between sites, the non-zero densities of each of the four major cohorts were pooled into a single data set and fit with modified versions of equations (22) and (23). The modifications consisted of inserting dummy variables for cohorts and the interactions of dummy variables with t-l, t or in(t). The same dummy variables that were used for otolith and morphometric analyses were used in these analyses. Preliminary analysis showed that it was not possible to produce a unique result when all nine cohorts were included in the pooled data set because nine dummy variables and their interactions produced a too-sparse data matrix; one that consisted primarily of 0s and 1s. Therefore, only the four major cohorts were included.

The version of the simple model [equation (22)] that explained the most variance in density (? = 0.83, n = 42) with all-significant parameters was

(24)

| variable | coefficient | SE | P |
|---|---|---|---|
| b0 | 9.209 | 0.6507 | <0.0001 |
| x3 | 3.840 | 0.6104 | <0.0001 |
| x7 | 1.334 | 0.5087 | 0.0127 |
| $(t+5)^{-1}$ | -13.7446 | 1.151 | <0.0001 |
| $x_3(t+5)^{-1}$ | -19.87 | 5.072 | 0.0004 |
| t+5 | -0.2252 | 0.0184 | <0.0001 |

where x3 = 1 for cohort 2 of Fairmount Island and O for the other four major cohorts, x7 = 1 for cohort 1 of Tatitlek Narrows and O for the other four cohorts, and age was transformed by the addition of 5 d in order to avoid negative ages due to the capture of larvae before the mean hatch dates. The fit of this model to the data is shown as curve A in Fig. 15. It clearly underestimates the density of 5-15 d old larvae and overestimates the density of 25-35 d old larvae.

The most important result of this simple model was the finding that a constant mortality of 0.23 $d^{-1}$ occurred in all four major cohorts and that there were no site effects that could be related to the presence or absence of oil. Equation (24) indicates that the highest initial density was measured for cohort 2 of Fairmount Island, that the initial density of cohort 1 of Tatitlek Narrows was significantly lower, and that both initial densities were higher than those of cohort 2 of Bass Harbor and cohort 2 of Rocky Bay. The latter two initial densities were not significantly

(P > 0.05) different from each other. Equation (24) also indicates that diffusive transport was significantly lower at the Fairmount Island site than at the other three sites.

One interesting result of this model is that it shows that the waves of newly-hatched larvae passed through the sampling stations 1 and 5 d after hatch. This supports the assumption that the ascending limb of the catch curve is caused by the time required for the larvae to be transported from the hatch sites to the plankton station 1-5 km offshore.

The second model explained 5% more variance ($r^2 = 0.88$, n = 42) than the first, and it provided a superior fit to the densities of young larvae (Fig. 15: Curve B)

(25)

| variable | coefficient | SE | P |
|---|---|---|---|
| $b_0$ | 29.284 | 2.141 | <0.0001 |
| x3 | 4.191 | 0.566 | <0.0001 |
| x7 | 1.920 | 0.601 | 0.0030 |
| $\ln(t+5)$ | -8.766 | 0.608 | <0.0001 |
| $(t+5)^{-1}$ | -33.78 | 2.32 | <0.0001 |
| $x_1(t+5)^{-1}$ | -11.93 | 2.20 | <0.0001 |
| $x_3(t+5)^{-1}$ | -41.81 | 5.76 | <0.0001 |
| $x_7(t+5)^{-1}$ | -22.43 | 6.79 | 0.0022 |

where $x_1$ = 1 for cohort 2 of Bass Harbor and O for all other cohorts. These results support the conclusion that mortality is not significantly different between sites. If we assume that horizontal variance due to diffusion increases with age to a power between 2 and 3, as Okubo (1971) reported in his review of dye experiments in the sea, then the mortality coefficient $\beta$, ranges from 5.77 to 6.77, which means that Z fell from 5.77-6.77 d-l at age 1 to 0.23-0.27 $d^{-1}$ at age 25 d and to 0.10-0.0.12 $d^{-1}$ at age 55 d. The second model also supports the conclusion that the Fairmount Island site supported the highest initial larval density, followed by Tatitlek Narrows and the two treatment sites.

The second model differs from the first in that it indicates that all four sites had significantly different rates of diffusion. In order of increasing diffusive transport they are: Fairmount Island, Tatitlek Narrows, Bass Harbor and Rocky Bay. These

differences are almost certainly related to the general counter-clockwise pattern of surface water circulation in the Sound, and not to oil contamination. Herring larvae that hatch near Hinchinbrook Entrance will tend to be transported northward at a faster rate than larvae that hatch near the northern shore of the Sound.

## 5. DISCUSSION

This study shows that growth and mortality of herring larvae in Prince William Sound in 1989 was not affected by the Exxon Valdez oil spill. There is no evidence that oil decreased the number of cohorts of larvae or their initial density in the treatment sites. Three cohorts were hatched at each of the four sites, which is the usual number observed at a spawning site, e.g. Lambert (1984), McGurk (1989a,b). Initial densities of larvae were much higher in the two northern control sites than the two treatment sites, but this is probably a consequence of a greater number of eggs laid in the two control sites, rather than a reflection of differences in egg mortality. McGurk et al. (1990) reported that herring eggs from the Fairmount area, the Naked Island archipelago and Rocky Bay that were incubated in the laboratory died at a rate of only $3\%\cdot d^{-1}$ for all sites. Since natural mortality of herring eggs, which includes predation, is usually much higher than this rate, e.g. as much as 90% over the incubation period [see review by Palsson (1984)], a difference in non-predation survival of a few percentage points due to oil would not have had a measurable effect on initial densities of herring larvae. We encourage other investigators to test this argument by comparing the density of herring eggs and the areal extent of herring spawn between treatment and control areas.

Population growth rates of all four sites were within the range observed for Atlantic and Pacific herring larvae, i.e. between 0.1 $mm\cdot d^{-1}$ and 0.5 $mm\cdot d^{-1}$ (Jones 1978, McGurk 1984b). All differences in growth between sites and cohorts were due to differences in water temperature; there was no evidence for an independent site effect. Similarly, there were no significant differences between sites in recent growth rates calculated from otolith ring widths. Age was the only factor that explained a significant amount of variance in recent growth rates. Neither morphometric condition or RNA-DNA ratios support the hypothesis that growth and condition was less in the oiled sites than in the control sites.

Mortality rates were not significantly different between sites, regardless of which model was used: constant orage-dependent. The average constant rate of O.23 $d^{-1}$ falls within the range reported for both Atlantic and Pacific herring larvae (McGurk 1984b, 1989a, b).

The finding of no obvious oil effect on growth and development of herring larvae is supported by the lack of differences in prey concentration and in the densities of non-herring fish larvae between sites. It indicates that the average concentration of hydrocarbons in the pelagic zone at the time the larvae hatched were probably too low to affect subsequent larval growth and mortality. Rice et al. (1987) reported a list of lethal doses for Pacific herring larvae exposed to the water-soluble fraction (WSF) of Cook Inlet crude oil: yolk sac larvae exposed from 16 h to 6 d had $LC_{50}$s of 2.8 to 2.3 ppm; feeding larvae exposed for 7 and 21 d had $LC_{50}$s of 1.8 and 0.36 ppm, respectively; and larval growth was significantly reduced after 7 d of exposure to 0.3 ppm. Therefore, it follows that hydrocarbon concentrations in the upper 30 m of the Prince William Sound were probably less than 0.3 ppm. We encourage other investigators to test this statement by reporting the concentrations of hydrocarbons measured from the pelagic zone of the Sound.

This argument is based on the assumption that the Tatitlek Narrows and Fairmount Island sites were completely free of any non-natural concentrations of hydrocarbons. It is a reasonable assumption given the fact that the oil slick never travelled near those sites. However, the assumption was never tested by chemical means. We understand that it may never be possible to know the 'hydrocarbon concentrations in the Fairmount Island site because few, if any, water samples were ever collected there in 1989 (Jeffrey Short, NOAA, Auke Bay Laboratory, Auke Bay, AK, pers. comm.). However, it maybe possible to test its status as a control site by measuring the activity of mixed-function oxygenase (MFO) enzymes of fishes captured there, specifically some of the frozen herring larvae that remain in cold storage.

## 6. RECOMMENDATIONS

### Herring Larvae Surveys

Since there is no evidence for an effect of oil on the population dynamics of herring larvae in the Sound, we do not recommend that a survey of larval herring in Prince

William Sound be repeated as part of the Exxon Valdez oil impact assessment program. Any future research on the early life history of herring in the Sound should be designed and conducted in order to answer questions related to fisheries ecology and management. These could include, but not be limited to, questions concerning the number of spawning stocks in the Sound and their degree of mixture; back-calculation of spawning stock biomass from larval densities; and the environmental factors controlling recruitment.

Of special relevance to this last point is the relationship between transport of herring larvae and the surface current patterns in the Sound. This report shows that larvae from the southern spawning grounds disperse at a faster rate than larvae from northern spawning grounds. Is this a response to current patterns? and if so, does this indicate that habitat for juvenile herring is concentrated in the northern half of the Sound?

Hydrocarbon Concentrations

We do not recommend reanalyzing the data of this study in order to include hydrocarbon concentrations measured from the water or from pelagic organisms because there are no differences in growth and mortality of herring larvae between sites that require an explanation based on oil contamination. Although such a comparison would be scientifically interesting, it is not directly relevant to the task of assessing the impact of the Exxon Valdez oil spill on herring resources of Prince William Sound.

However, we do recommend that hydrocarbon concentrations measured in the Sound should be compared to mixed function oxygenase (MFO) enzyme activity of the remaining frozen herring larvae that are stored by Microtek Ltd. Payne et al. (1987) has shown that MFO enzyme activity is a very sensitive indicator of exposure to hydrocarbons. The purpose of this proposed work would be to determine if the detoxification system of the larvae was stimulated by the hydrocarbons present in the pelagic zone of the Sound in May-June, 1989, or whether the concentration of hydrocarbons was too low to provoke a biochemical response. If the former is the case, then we may conclude that the detoxification system protected herring larvae and this was part of the explanation for the absence of an effect of oil on growth and

mortality. If the latter is true, then we may conclude that the larvae were never exposed to sufficient levels of hydrocarbons to threaten their growth or survival.

Cytogenetic Analyses of Herring Larvae

We recommend that some of the formalin-preserved larvae should be examined for the presence of cytogenetic abnormalities such as disruptions of the mitotic bundles. The purpose of this work would be to confirm that there were no sublethal effects of oil that were not expressed as growth or mortality.

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# Appendix A. Comparison of Methods of RNA-DNA Analysis

## A1. INTRODUCTION

The oldest method used to measure the concentrations of RNA and DNA in marine fish larvae is the Schmidt-Thannhauser (1945) technique modified by Munro and Fleck (1966) (Buckley 1979, 1984, Wright and Martin 1985, Fukuda et al. 1986, Buckley and Lough 1987, and Clemmessen 1987). It is based on the absorbance of UV light in the 260 nm band by purified nucleic acids. It has the disadvantage of requiring a minimum of 800 $\mu$g dry weight·sample$^{-1}$, which means that the nucleic acid concentrations of individual Prince William Sound herring larvae < 17 mm long could not be measured with this method. A technique for measuring amounts as small as 0.05 $\mu$g·mL$^{-1}$ of DNA and 0.1 $\mu$g·mL$^{-1}$ of RNA was introduced by LePecq and Paoletti (1966) and modified by Karsten and Wollenberger (1972, 1977). Prasad et al. (1972) showed that it produced results comparable to those of the Schmidt-Thannhauser method, at least for mammalian tissues. It was used most recently by Robinson (1988) and Robinson and Ware (1988) to measure RNA-DNA ratios of Pacific herring larvae of Georgia Strait, British Columbia. We used Karsten and Wollenberger's (1972, 1977) method as modified by Robinson (1988) on 270 herring larvae from four sites in Prince William Sound, but found that it produced a large number of very low RNA-DNA ratios, which suggested that the method was not giving accurate measurements of one or both kinds of nucleic acids. This appendix reports the experiments we carried out in order to determine which factor(s) were responsible for these unexpected results, and the method by which we corrected these data.

There were three possible sources of error. The RNA standard may have been contaminated with DNA, thereby inflating the slope of the standard curve and leading to underestimates of RNA concentration and underestimates of the RNA-DNA ratio. Contamination by small concentrations of DNA can lead to large errors in the RNA standard curve because DNA has a fivefold greater flourescence yield than RNA. Both Bentle et al. (1981) and Robinson (1988) reported that their RNA standards had to be corrected for contamination with DNA. This possibility was investigated by testing the purity of the RNA standard.

The second possibility was inhibition of RNAse leading to underestimation of RNA concentration and of the RNA-DNA ratio. This was investigated by using Bentle et al.'s (1981) method, which is a modification of Karsten and Wollenberger (1972, 1977) method that measures nucleic acid concentration through the loss of flourescence occurring after the sequential addition of DNAse and RNAse to the same reaction mixture. The flourescence remaining after addition of both enzymes represents background flourescence due to undigested RNA plus compounds other than nucleic acids.

The third possibility was that unknown compounds in herring larvae were flourescing thereby inflating the estimate of DNA concentration. Clemmessen (1988) recently described a method for measuring small quantities of nucleic acids that excludes this possibility by purifying nucleic acids of all cellular debris that could affect flourescence.

Our experimental design was based on comparing the RNA-DNA ratios of herring larvae collected from one control site, Tatitlek Narrows, in order to avoid confounding the results with a site effect or an oil effect. A total of 159 larvae were analysed: 71 with Karsten and Wollenberger's (1972, 1977) method, 58 with Clemmesen's (1981) method and 30 with Bentle et al.'s (1981) method. A wide range of dates and larval lengths were chosen for each group in order to account for date- and size-related effects.

## A2. METHODS

### Karsten and Wollenberger's (1972, 1977) method

In the first phase of this analysis whole-body concentrations of RNA and DNA were measured for 270 herring larvae that were chosen randomly from each of the 28 combinations of four sites and seven dates. This data is shown in Appendix J. Karsten and Wollenberger's (1972, 1977) method as modified by Robinson (1988) was used for the analysis. The technique is based on the enhanced flourescence of nucleic acids after introduction of the dye ethidium bromide. For this study, each individual larva was thawed and measured for standard length to the nearest 0.5 mm. The larva was then placed in 3 mL of ice-cold phosphate-buffered saline (PBS) and homogenized at 25,000 rpm for two 15 s treatments. Two replicates of 0.5 mL

aliquots were taken and processed for determination of DNA. This involved adding 0.5 mL of heparin plus 0.5 mL of RNAse in order to digest all of the RNA leaving only DNA. Two replicates were processed for total nucleic acids, which meant adding ethidium bromide and heparin and measuring total flourescence. One replicate was used to measure the flourescence background, no ethidium bromide was added to that aliquot. One blank aliquot (PBS but no herring larvae) was run each day in order to calibrate the flourometer. The difference between the mean concentration of total nucleic acids and the mean concentration of DNA was the concentration of RNA. Robinson (1988) modified this method by increasing the time that the DNA aliquots were incubated with RNAse from 20 to 30 min, and by increasing the concentration of RNAse from 50 to 100 $\mu g \cdot mL^{-1}$. These modifications were added in order to increase confidence that all RNA would be digested by the RNAse. The flourometric response to known concentrations of nucleic acids (i.e. standard curves) was measured in triplicate at eight concentrations of DNA (0.05, 0.1, 0.25, 0.5, 1, 2, 3, and 4 $\mu g \cdot mL^{-1}$) and six concentrations of RNA (0.25, 1, 3, 5, 10, and 15 $\mu g \cdot mL^{-1}$). The DNA standard was Sigma number D6898 and the RNA standard was Sigma number R7250.

Purity of RNA Standard

In the second phase of analysis we tested the purity of the RNA standard in five ways:

(1) digesting it with RNAse/PBS and measuring flourescence. A pure sample would exhibit no residual flourescence;

(2) digesting it with DNAse/PBS and measuring flourescence. A pure sample would exhibit no change in flourescence;

(3) digesting it with RNAse/buffer C and measuring flourescence. A pure sample would exhibit no change in flourescence;

(4) measuring the concentration of DNA in the RNA standard using a dye specific for DNA - bisbenzimidazole. A pure sample would not flouresce; and

(5) adding 10 $\mu$g of DNA to the RNA standard and measuring the concentration of DNA using bisbenzimidazole. A pure sample would flouresce by an amount equivalent to 10 $\mu$g of DNA.

We used DNAse rather than alakali hydrolysis to digest any DNA that was present in the RNA standard because DNAse is more specific to DNA than alkali hydrolysis.

Bentle et al.'s (1981) Method

In the third phase of analysis we measured nucleic acid concentrations of a subset of 30 larvae from the Tatitlek Narrows site using Bentle et al.'s (1981) method. This method also depends on the enhanced flourescence of ethidium bromide-nucleic acid complexes, but a different ionic medium is used in order to optimize the activity of DNAse when it is used in combination with RNAse. Each larva was thawed, measured for standard length to the nearest 0.1 mm and homogenized in 3 mL of ice-cold buffer C, instead of PBS. Buffer C consisted of 20 mM Tris-HCl at a pH of 7.5, 1 mM MgCl, 0.8 nM CaCl and 50 mM NaCl. Ethidium bromide was added to two aliquots and the mean concentration of total nucleic acids was measured flourometrically. Then, RNAse to a final concentration of 25 $\mu g \cdot mL^{-1}$ was added to two other aliquots in order to digest all RNA and give a concentration of DNA. Finally, RNAse plus DNAse I to a final concentration of 5 $\mu g \cdot mL^{-1}$ was added to another two aliquots in order to digest all nucleic acids and give a background flourescence. Volumes of aliquots and incubation times were the same as in the first stage of analysis. A separate set of standard curves were run for this method.

Clemmessen's (1988) Method

In the fourth phase of analysis 58 herring larvae were analysed with Clemmesen's (1988) method. This method differs from the previous two methods in two ways: nucleic acids of fish larvae were purified of all compounds that may alter flourescence by repeatedly washing the homogenized fish with organic solvents; and a DNA-specific dye, bisbenzimidazole, was used instead of ethidium bromide to measure DNA concentration. Each larva was thawed and standard length was measured to the nearest 0.1 mm. The larva was homogenized in an ice-cold buffer

containing 0.3 mL of 0.05 M Tris-HCl, 0.1 M NaCl, 0.01 M EDTA at a pH of 9.0, and 0.2 $mg \cdot mL^{-1}$ of proteinase K The homogenate was centrifuged at 6,000 rpm for 15 min and then the supernatant was decanted into a new vial into which 0.3 mL of 80% phenol and 0.3 mL of chloroform/isoamylalcohol (24:1) were added. The solution was mixed for 10 min and then centrifuged at 6,000 rpm for 10 rein, The phenol-chloroform/isoamylalcohol phase was discarded and the aqueous phase containing the nucleic acids was washed a second time. The aqueous phase from that washing was extracted, combined with 0.3 mL of the chloroform/isoamyalcohol mixture, mixed for 1 min and then centrifuged for 5 min. This procedure was repeated a second time in order to completely wash the aqueous phase and prepare it for measurement of nucleic acid concentration Finally, 0.2 mL of buffer was added to the aqueous phase and it was separated into several aliqots. Ethidium bromide was added to one aliquot and total nucleic acid concentration was measured from its flourescence. Bisbenzimidazole was added to another aliquot and DNA concentration was measured flourometrically. RNA concentration was calculated as the difference between total nucleic acid and DNA concentration. There was sufficient material to allow two measurements of total nucleic acid concentration and DNA concentration for each fish. A standard curve for DNA was used, and the fluorometer was blanked everyday.

Methods of Comparison

Nucleic acid concentrations were compared between methods using general linear models with dry weight as a covariate. Weight was a more acccurate measure of size than length because yolk sac larvae were the shortest larvae but were actually heavier than small non yolk sac larvae. Dry weight was calculated from larval length using the logistic weight-length equation shown in Fig. 11. An additional 22 $\mu$g of dry weight was added to yolk sac larvae. This was the mean@ weight (SD = 11) of 20 larvae whose yolk sac dimensions had been measured. Julian date of larval collection and average water temperature in the upper 30 m were included as auxiliary variables. Prey concentration was not included as an auxillary variable because it was highly correlated with larval size having been derived from larval length. Weight, DNA, RNA, and the RNA-DNA ratio were in-transformed in order to normalize their distributions.

Yolk Sac vs Non Yolk Sac Larvae

**Before comparing nucleic acid concentrations between groups of larvae it was necessary to determine whether yolk sac larvae could be pooled with non yolk sac larvae. Were there significant differences in concentration of nucleic acids per $\mu$g dry weight between yolk and non-yolk tissue? The concentrations of RNA and DNA in the yolk and non-yolk tissues of ten larvae were measured with Clemmessen's (1988) method. The length and height of each yolk sac was measured in order to calculate yolk sac volume using the equation for an ellipsoid [equation (5)] and then the sac, including its epitheliums was removed with a dissecting pin. Dry weight of the yolk sac was calculated from yolk sac volume by assuming a specific gravity of1 $g \cdot cm^{-3}$ and an 80% water content or 200 $\mu g \cdot mm^{-3}$. RNA and DNA concentrations ($\mu$g per yolk sac or per larva) were divided by dry weight of the yolk sac or of the larva in order to remove the effect of size. These new concentrations, $\mu$g nucleic acid per $\mu$g dry weight of larval or yolk tissue, were in-transformed in order to normalize their distributions and then compared between body compartments with one-way analysis of variance (ANOVA).**

## A3. RESULTS AND DISCUSSION

### A3.1 Contamination of Standards

**The weight of evidence supports the conclusion that the RNA standard was pure and that no correction for DNA contamination was necessary. Four of the five tests shown in Table Al support this conclusion including Clemmessen's (1988) method, which is the most accurate and precise of the three methods examined in this study. The incubation of RNA with RNAse/PBS caused complete digestion of RNA in three replicate samples. The absence of residual flourescence indicated that no other nucleic acids were present. Similar results were obtained when buffer C was used instead of PBS. These results were supported by using Clemmesen's (1988) method to analyse the RNA standard: it did not produce any flourescence of the DNA-specific dye, bisbenzimidazole. In order to see if Clemmessen's (1988) method was able to detect small quantities of DNA, we added 10 $\mu$g DNA to the RNA standard and repeated the test. Flouresence equivalent to 10 $\mu$g of DNA was measured, confirming the high sensitivity of the method. In contrast to these results, the incubation of the RNA standard with DNAse/PBS caused a partial loss of**

**TABLE A1**

**Tests for contamination of RNA standard (Sigma #R7250) by DNA. Predicted response assumes pure RNA.**

| Treatment | | Predicted | Measured |
|---|---|---|---|
| 1. | Incubation of RNA standard with RNAse-PBS. | complete loss of fluorescence | complete loss of fluorescence |
| 2. | Incubation of RNA standard with DNAse-PBS. | no change in fluorescence | 50% loss of fluorescence |
| 3. | Incubation of RNA standard with RNAse-buffer C. | complete loss of fluorescence | complete loss of fluorescence |
| 4. | Measurement of DNA concentration in RNA standard with DNA - specific dye (Clemmessen 1988). | no fluorescence | no fluorescence |
| 5. | Measurement of 10 $\mu$g DNA added to RNA standard with DNA - specific dye (Clemmessen 1988). | fluorescence equivalent to 10 $\mu$g DNA | fluorescence equivalent to 10 $\mu$g DNA |

flourescence, which appeared to indicate the presence of DNA. Fig. Al shows the standard curves for RNA with and without the addition of DNAse/PBS; the latter slope is 2.39 times higher than the former slope.

We have no explanation for the anomolous results of the DNAse/PBS treatment. It can be hypothesized that they were due to contamination of DNAse with RNAse. We used high concentrations of DNAse, 20 $\mu g \cdot mL^{-1}$, in order to digest any DNA that may have been present in the RNA standard. If the DNAse was contaminated with RNAse, then there may have been sufficient RNAse to cause the observed loss in flourescence. This would not have affected the results of RNA-DNA measurements made with Karsten and Wollenberger's (1972, 1977) and Clemmessen's (1988) method because DNAse is not used in either method. However, it would have affected the results made with Bentle et al.'s (1981) method because DNAse is added to the reaction mixture before RNAse. It would cause an overestimation of DNA concentration, an underestimation of RNA concentration and an underestimation of the RNA-DNA ratio because the RNAase-contaminated DNAse would cause a greater loss of flourescence than was justified by the amount of DNA present.

We have no explanation why Bentle et al. (1981) and Robinson (1988) observed partial loss of flouresence of their RNA standard after alkali hydrolysis.

**A3.2 Comparison of Methods**

Comparison of Yolked and Non-Yolked Larvae

Table A2 shows the ranges and arithmetic mean@ 1 SD) sizes and RNA and DNA concentrations of the ten larvae whose nucleic acid concentrations were measured for the yolk sac and the body separately with Clemmessen's (1988) method. The geometric mean DNA concentration of yolk, 0.006 $\mu g \cdot (\mu g \text{ dry weight})^{-1}$, was significantly ($0.001 < P$ c $0.01$) higher than the geometric mean DNA concentration of the body, 0.003 $\mu g \cdot (\mu g \text{ dry weight})^{-1}$. The geometric mean RNA concentration of the yolk, 0.029 $\mu g \cdot (\mu g \text{ dry weight})^{-1}$, was also significantly ($0.02 < P < 0.05$) higher than the geometric mean RNA concentration of the body, 0.016 $\mu g \cdot (\mu g \text{ dry weight})^{-1}$. Although these differences did not translate into significant ($P > 0.05$) differences

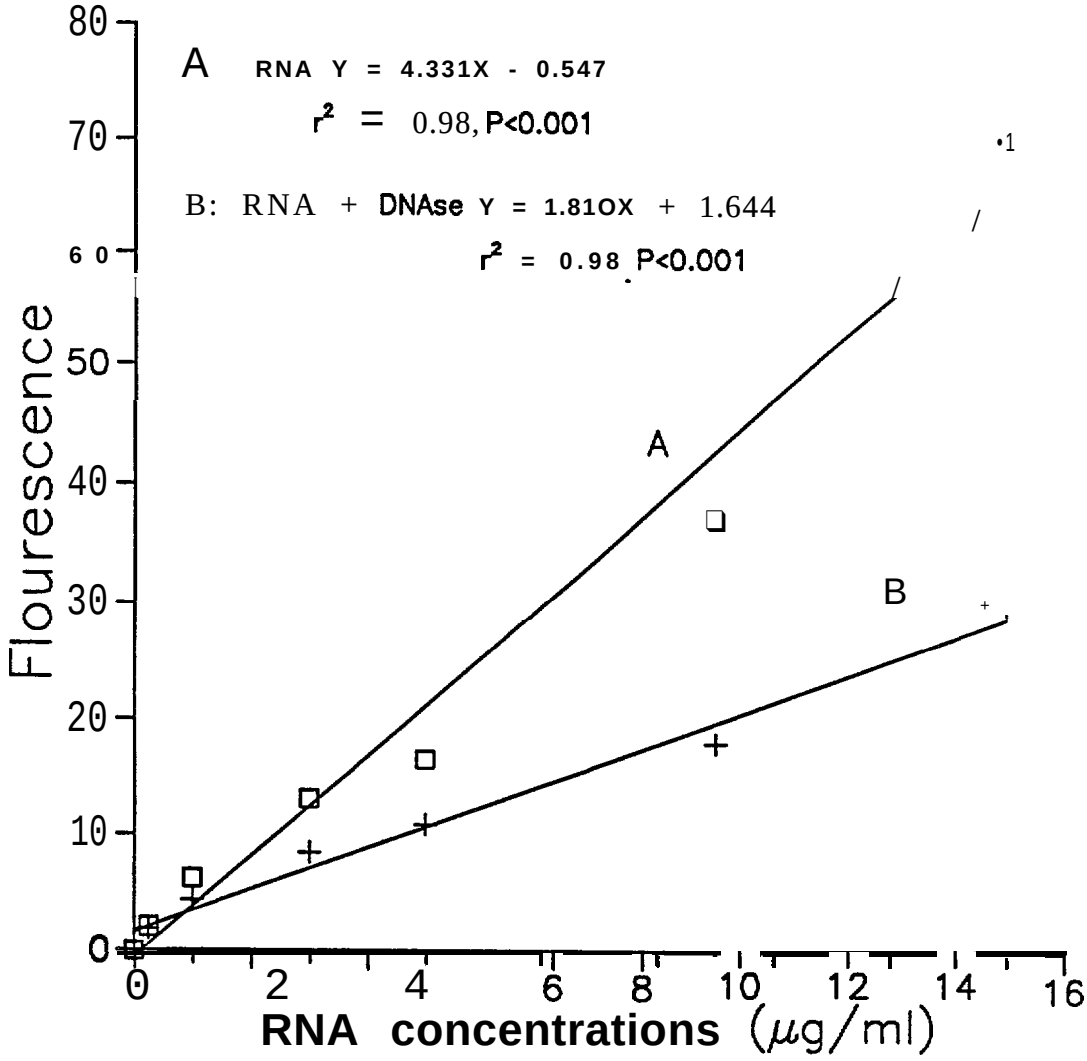


**Figure A1.** Standard curves for RNA and for RNA plus DNAse 1.

**TABLE A2**

Size and nucleic acid concentrations of the yolk and non-yolk compartments of 10 larvae measured with Clemmessen's (1988) method. Star indicates a significant (0.02 <P<0.05) difference between mean geometric DNA and RNA concentrations of yolk sac and body.

| | Larval length (mm) | Dry weight (μg) | | | DNA (μg fish$^{-1}$) | | | RNA (μg fish$^{-1}$) | | | RNA/DNA | | | DNA (μg/μgW) | | RNA (μg/μgW) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | larvae | yolk | total | larvae | yolk | total | larvae | yolk | total | larvae | yolk | total | larvae | yolk | larvae | yolk |
| minimum | 6.5 | 135 | 13 | 156 | 0.215 | (3.080 | .0305 | 1.460 | 0.135 | 2.130 | 2.496 | 0.370 | 2.278 | 0.001 | 0.002 | 0.010 | 0.009 |
| maximum | 8.2 | 145 | 42 | 186 | 0.605 | 0.365 | 0.965 | 3.360 | 1.545 | 4.340 | 14.000 | 15.737 | 11.469 | 0.004 | 0.024 | 0.024 | 0.058 |
| mean | 7.4 | 141 | 25 | 166 | 0.393 | 0.236 | 0.544 | 2.273 | 0.865 | 3.138 | 6.690 | 7.983 | 6.664 | 0.003 | 0.007* | 0.016 | 0.040* |
| SD | 0.5 | 4 | 11 | 11 | 0.149 | 0.266 | 0,202 | 0.566 | 0.512 | 0.842 | 3.271 | 6.000 | 3.169 | 0.001 | 0.006 | 0.004 | 0.032 |

in RNA-DNA ratios, yolk sac and non yolk sac larvae were treated as separate populations in subsequent analyses.

Comparison of Methods

**Two-way ANOVA showed that dry weight of larvae varied significantly ($P < 0.001$) with date, but not with method or the interaction of date and method. This meant that weight could be used as an independent covariate for comparing nucleic acid concentrations obtained from the three methods. Weight was preferred over date because the samples from Tatitlek Narrows were-mixtures of three separate cohorts (i.e. size-classes) of larvae.**

**The general linear model that explained the most variance (? = 0.91, n = 159) in ln(DNA) with all-significant parameters was:**

| (Al) variable | coefficient | SE | P |
|---|---|---|---|
| constant | -2.1572 | 0.5759 | 0.0003 |
| ln(weight) | 0.6730 | 0.0608 | <0.0001 |
| $g_1$ | 1.5819 | 0.0639 | <0.0001 |
| $g_3$*yolk | -0.3554 | 0.1230 | 0.0044 |
| date | -0.0385 | 0.0110 | 0.0006 |
| temperature | 0.5988 | 0.1284 | <0.0001 |

**where $g_1$ is a dummy variable with a value of 1 for Karsten and Wollenberger's (1972, 1977) method and a value of O for all other methods, g2 has a value of 1 for Bentle et al.'s (1981) method and O for all other methods, $g_3$ has a value of 1 for Clemmessen's (1981) method and O for all other methods, and the dummy variable 'yolk' has a value of 1 for yolk sac larvae and O for non-yolk sac larvae. Fig. A2 shows the fit of the model to the data. The non-linear shape of the predicted DNA concentration is due to differences in water temperature between dates.**

**The model indicates that DNA concentration was greater for Karsten and Wollenberger's (1972, 1977) method that for the other two methods. It also shows that DNA concentration was lower in yolk sac larvae than in non yolk sac larvae.**

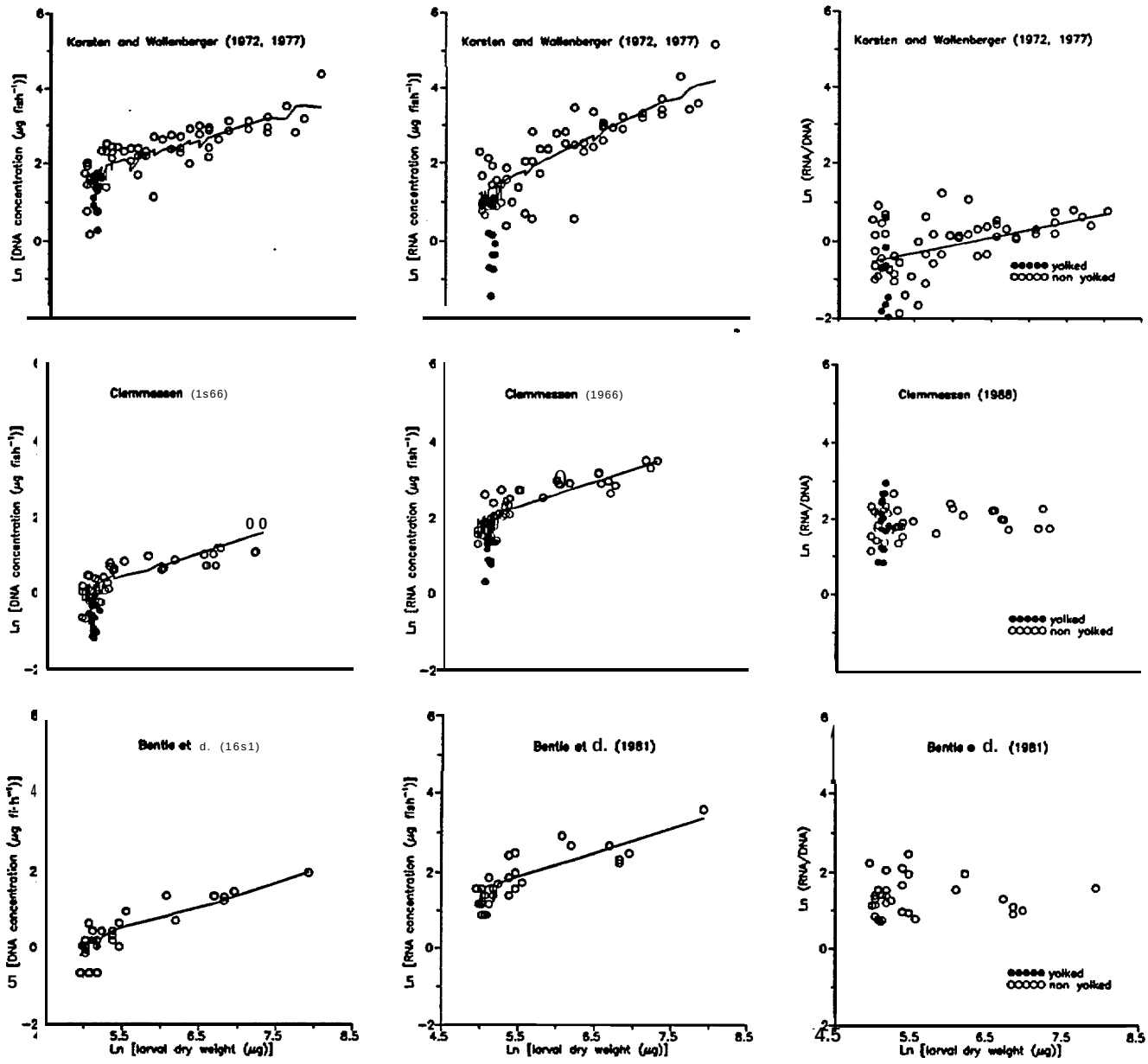


**Figure A2.** **Plots of DNA concentration, RNA concentration, and the ratio of RNA to DNA of herring larvae against weight of larvae for each of three different methods of RNA-DNA analysis. Solid lines are from equations (Al), (A2) and (A3) respectively.**

The general linear model that explained the most variance in ln(RNA) (? = 0.80, n = 159) was:

| (A2) variable | coefficient | SE | P |
|---|---|---|---|
| constant | -3.7237 | 0.8489 | <0.0001 |
| ln(weight) | 1.0060 | 0.0907 | <0.0001 |
| $g_2$ | 2.3135 | 0.8021 | 0.0045 |
| $g_3$ | 2.4971 | 0.7534 | 0.0012 |
| $g_2$*ln(weight) | -0.3981 | 0.1397 | 0.0050 |
| $g_3$*ln(weight) | -0.3512 | 0.1311 | 0.0082 |
| $g_1$*yolk | -0.8708 | 0.1914 | <0.0001 |
| date | -0.0330 | 0.0142 | 0.0214 |
| temperature | 0.6276 | 0.1624 | 0.0002 |

This model shows that RNA concentrations were significantly higher with Clemmessen's (1988) method than with the other two methods.

The general linear model that explained the most variance in ln(RNA/DNA) (? = 0.78, n = 159) was:

| (A3) variable | coefficient | SE | P |
|---|---|---|---|
| constant | -2.5182 | 0.5543 | <0.0001 |
| $g_2$ | 3.8883 | 0.5650 | <0.0001 |
| $g_3$ | 4.4200 | 0.5599 | <0.0001 |
| $g_1$*ln(weight) | 0.4023 | 0.0921 | <0.0001 |
| $g_1$*yolk | -0.9538 | 0.2002 | <0.0001 |

This model shows that the RNA-DNA ratio measured with Clemmessen's (1988) method was about 1.5 times higher than the ratios measured with Bentle et al.'s (1981) method, and 5-17 times higher than the ratios measured with Karsten and Wollenberger's (1972, 1977) method (Fig. A2). The RNA-DNA ratios measured with Clemmessen's (1988) and Bentle et al.'s (1981) methods did not vary with length. These results are independent of temperature or date of collection.

These results indicate that the phenomenon of "quenching" of flourescence that was briefly discussed by Clemmessen (1988) was affecting nucleic acid concentrations

measured with Karsten and Wollenberger's (1972, 1977) and Bentle et al.'s (1981) methods. It inflated the measurement of DNA concentration in Karsten and Wollenberger's (1972, 1977) method by a factor of 4 to 5 and deflated RNA concentration by a factor of 1 to 3. This produced RNA-DNA ratios for this method that were at least 4 to 15 times lower than the other two methods.

There are several possible mechanisms of quenching including inhibition of RNAse, flourescence of compounds other than nucleic acids, and contamination of DNAse with RNAse. In Clemmessen's (1988) method these factors were removed by purifying the homogenized larva, by using bisbenzimidazole to measure DNA flourescence, and by not using either DNAse or RNAse. Although purification was not part of Bentle et al.'s (1981) method, inhibition of RNAse or residual flourescence would not have affected measurement of DNA concentration because it was measured by adding DNAse to the homogenate. However, it would have affected measurement of RNA concentration. Regardless of the mechanism of quenching, the result would be the same - the production of a block of unexplained flourescence that must be added or subtracted from RNA or DNA. The amount of flourescence remaining after the addition of both DNAse and RNAse was measured in the 30 larvae processed with Bentle et al.'s (1981) method. Expressed as a percentage of total flourescence for nucleic acids for each fish, it ranged from 40 to 72.4% with a mean of 60.8% (SD = 8.4). It was not significantly correlated with larval length, RNA and DNA concentrations or the ratio of RNA to DNA concentration. It was not possible to compare background flourescence between yolked and non-yolked larvae because only one of" the 30 larvae was yolked. However, equations (Al) to (A3) clearly show that yolk was an important source of background flourescence.

Accuracy of Methods

Clemmessen's (1988) method was the most accurate method and Karsten and Wollenberger's (1972, 1977) method was the least accurate method. Over 50% of the RNA-DNA ratios estimated with Karsten and Wollenberger's (1972, 1977) method were below 1.0. These ratios are too low to be acceptable because they indicate massive starvation, a phenomenon that is not compatible with the results of the other two methods of nucleic acid analysis, or with other evidence on growth and condition collected in this study, or with the fact that some of the 'starving' fish

were over 15 mm long. Such large larvae were unlikely to be starving given the relatively high prey concentrations measured in Prince William Sound, and given the fact that the larvae were large enough to have successfully ascended the learning curve for foraging. The primary cause of the low ratios measured by Karsten and Wollenberger's (1972, 1977) method was an overestimation of DNA concentration by a factor of 4 to 5 times. The secondary cause was an underestimation of RNA concentration by a factor of 1 to 3 times.

The difference in accuracy is calculated from equation (A3): for an 8.0 mm long larvae with no yolk sac the mean ln(RNA-DNA ratio) was 0.549 for Karsten and Wollenberger's (1972, 1977) method, 4.041 for Bentle et al.'s (1981) method and 6.576 for Clemmessen's (1988) method. Thus Clemmessen's (1988) method is 12.0 times more accurate than Karsten and Wollenberger's (1972, 1977) method and 1.63 times more accurate than Bentle et al.'s (1981) method.

Precision of Methods

Clemmessen's (1988) method is more precise than Bentle et al.'s (1981) method, and both are much more precise than Karsten and Wollenberger's (1972, 1977) method. This rank order was based on the standard error (SE) of the intercept of a separate linear regression of ln(RNA-DNA ratio) on ln(weight) for each method. The SE was 0.4417, 0.4887 and 0.7939 for Clemmessen's (1988), Bentle et al.'s (1981) and Karsten and Wollenberger's (1972, 1977) method, respectively. This is shown graphically in Fig. A2; the scatter of ln(RNA-DNA ratio) about the regression model was least for Clemmessen's (1988) method and most for Karsten and Wollenberger's (1972, 1977) method.

c orrection of RNA-DNA Ratios

The relatively high $r^2$ of equations (Al) and (A2) makes it possible to correct the RNA and DNA concentrations measured with Karsten and Wollenberger's (1972, 1977) method and Bentle et al.'s (1981) method to values approximating those that would have been measured if Clemmessen's (1988) method had been used on all larvae. Correction factors for DNA and RNA concentrations were calculated from the coefficients of equations (Al) and (A2) and are shown in Table A3. The corrected RNA-DNA ratios were then calculated from the corrected concentrations.

RNA-DNA ratios of yolk sac larvae were corrected using the same factors used for non yoik sac larvae.

TABLE A3

Factors for correcting RNA and DNA concentrations measured with Karsten and Wollenberger's (1972, 1977) and Bentle et al.'s (1981) methods. The factor is multiplied by the original measurement to obtain the corrected measurement.

| Method | DNA | RNA |
|---|---|---|
| Karsten and Wollenberger (1972, 1977) | 0.2056 | $12.1472W^{-0.3512}$ |
| Bentle et al. (1981) | 0.7009 | $1.2015W^{0.0469}$ |

Note: W = larval dry weight.

## Appendix B. Corrections of Herring Density for Net Avoidance

It is well known that fish larvae evade towed plankton nets and that evasion increases with increasing size of larvae (Smith and Richardson 1977). Therefore, although newly-hatched herring larvae are probably completely vulnerable to capture with the 60 cm diameter bongo nets used in this study, the densities of larger larvae must be corrected for evasion.

Night/day catch ratios are not available for this study because no night catches were taken in Prince William Sound. McGurk (1989a) reported night/day catch ratios for Pacific herring larvae from Bamfield Inlet, B.C., but it is uncertain whether they are applicable to this study because they were based on catches taken with a different kind of gear: a 40 cm diameter bongo net with 471 $\mu$m mesh. There is also some question whether night/day catch ratios are sufficient to account for all evasion because avoidance of nets by large Pacific herring larvae occurs at night as well as during the day (McGurk 1989a).

In this report, I adopted the method used by Ware and Lambert (1985) to correct the densities of Atlantic mackerel, Scomber scombrus, larvae for net evasion. This method is based on a model of the probability of capture, p, proposed by Clutter and Anraku (1968)

$$(B1)\quad p = 1 - \frac{1}{\pi}\left[\frac{a}{R^2}(R^2 - a^2/4)^{1/2} + 2R^2\sin^{-1}(a/2R))\right]$$

where R = radius of net (mm) and a = distance (mm) a larva moves between the time it reacts to a net and the time the net reaches its plane. This distance is the product of the escape speed of a larva, $U_{max}$ (mm.s$^{-1}$), and the time it has to avoid the net, $t_r$ (s), once it has detected it, i.e.

$$(B2)\quad a = t_r U_{max}.$$

Escape Speed of Herring Larvae

$U_{max}$ of Atlantic herring larvae increases with larval size and water temperature, and it follows a dome-shaped relationship with number of days of starvation, increasing over the first four days of starvation and then decreasing afterward (Bailey 1984, Bailey and Batty 1984, Yin and Blaxter 1987). Preliminary multiple regression analysis showed significant differences between these three sets of data which were correlated with temperature. However, the escape speeds predicted by this analysis for temperatures below $9\,^{\circ}C$ were too low to be realistic, indicating that the speed-temperature relationship developed from the narrow temperature range of 9-11°C could not be extrapolated to the relatively low temperatures of 5-7°C measured in Prince Wiliam Sound. Therefore, we adopted the method used by Ware and Lambert (1985): the length-and starvation-dependence of escape speed was estimated from one set of data collected at one temperature, and then an assumed $Q_{10}$ rate was used to extrapolate a temperature effect.

Yin and Blaxter (1987) reported the most complete set of escape speeds of Atlantic herring larvae (Table Bl). Multiple regression showed that 71910 of the variance in $U_{max}$ $(mm \cdot s^{-1})$ could be explained by

| | | | | |
|---|---|---|---|---|
| (B3a) | $\ln(U_{max})$ = | 1.4694 + | 1.1499ln(L) - | $2.36 \times 10^{-3} N_S^2$ |
| | (SE) | (0.2469) | (0.1000) | $(0.548 \times 10^{-3})$ |
| | (P) | (<0.0001) | (<0.0001) | (0.0001) |

or

(B3b) $U_{max} = 4.35\exp(-2.36 \times 10^{-3} n_s^2) L^{1.15}$

where L = length (mm) of larvae and $n_s$ = duration (d) of period of starvation.

**Table B1.**

**Mean effective escape speeds of Atlantic herring larvae reported by Yin and Blaxter (1987). Water temperature was 8-9°C. Stimulus for escape was either the touch of a wire probe or an attempt to suck them into a glass pipette.**

| | Stimulus = probe | | Stimulus = pipette | |
|---|---|---|---|---|
| Length (mm) | Escape speed ($mm.s^{-1}$) | Number of days starved | Escape speed ($mm.s^{-1}$) | Number of days starved |
| 8.0 | 54 | 0 | 57 | 0 |
| 8.3 | 55 | 2 | 59 | 2 |
| 8.6 | 59 | 4 | 65 | 4 |
| 8.7 | 54 | 8 | 57 | 8 |
| 8.7 | 40 | 10 | 48 | 10 |
| 8.7 | | | 45 | 12 |
| 8.8 | 55 | 6 | 58 | 6 |
| 9.1 | 29 | 0 | | |
| 9.4 | 44 | 0 | 57 | 0 |
| 9.7 | 53 | 0 | 65 | 0 |
| 10.0 | 58 | 2 | 67 | 2 |
| 10.2 | 66 | 4 | 72 | 4 |
| 10.3 | 50 | 6 | 55 | 6 |
| 10.3 | | | 37 | 14 |
| 10.4 | 51 | 8 | 61 | 8 |
| 10.4 | 43 | 10 | 51 | 10 |
| 10.4 | 19 | 12 | 48 | 12 |
| 13.4 | 52 | 0 | 67 | 0 |
| 14.5 | 76 | 2 | 91 | 2 |
| 14.5 | 82 | 4 | 100 | 4 |
| 14.5 | 81 | 6 | 95 | 6 |
| 14.5 | 72 | 8 | 91 | 8 |
| 14.5 | 55 | 10 | 80 | 10 |
| 14.5 | 50 | 12 | 76 | 12 |
| 19.0 | 106 | 2 | 138 | 2 |
| 19.0 | 126 | 4 | 144 | 4 |
| 19.0 | 121 | 6 | 141 | 6 |
| 19.0 | 117 | 8 | 134 | 8 |
| 19.0 | 111 | 11 | 118 | 11 |
| 19.0 | 90 | 13 | 109 | 13 |

If a $Q_{10}$ of 2 is assumed, then the intercept is doubled from 4.35 to 8.69. A two-point regression describes this increase as

(B4) intercept = $2.37\exp(0.066T)$

where T = temperature (°C). Substituting equation (B4) into equation (B3b) gives

(B5) $U_{max} = 2.37\exp(0.066T - 2.36\times10^{-3}n_s^2)L^{1.15}$.

Time to React

Ware and Lambert (1985) argued that fish larvae probably react to pressure waves extending up to 1.5 m in front of a towed net. Therefore, $t_r$ should be independent of the size of fish. If a net is towed at a speed of 1-3 $m\cdot s^{-1}$, then $t_r$ should be approximately 1-2s. A more accurate way of calculating $t_r$ is to note that p goes to zero as a approaches the diameter of the net (= 2R) (Ware and Lambert 1985). Thus,

(B6a) $2R = t_r U(L_{max})$

where $U(L_{max})$ = escape speed for $L_{max}$, the length (mm) of the largest larvae captured in the study, and

(B6b) $t_r = 2R/U(L_{max})$.

Examination of the catch curve for the pooled samples of this study shows that $L_{max}$ was about 24 mm (Fig. B1). The average water temperature during the study was 6.9°C, and all large larvae are assumed to be successfully feeding, i.e. $n_s$ = O. Therefore, $U_{max}$ predicted from equation (B5) is 145 $mm\cdot s^{-1}$, and $t_r$ is predicted from equation (B6b) to be 4s. This value of $t_r$ was used in all corrections of larval densities. Table B2 shows that the correction factors (= l/p) calculated from equation (B1) ranged from 1.2 in newly-hatched larvae to 1.5 in large larvae.

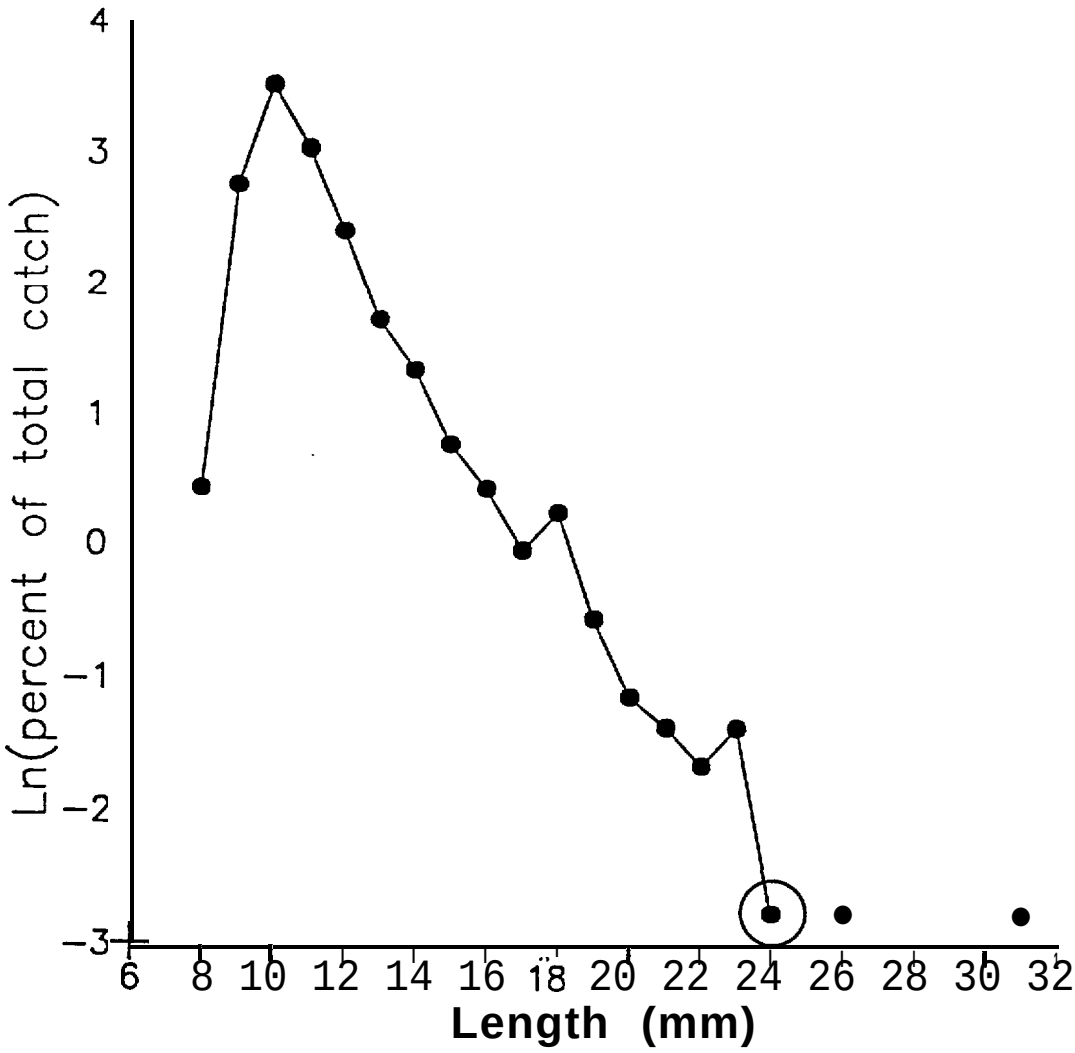


**Figure B1.** **Catch curve of herring larvae, combining all samples. The circled point is $L_{max}$, the largest larvae that could be captured. Larvae** longer than $L_{max}$ were incidental catches.

Table B2. Number and density of herring larvae In Prince William Sound **in** 1989.

| Date | Cohort 1 no. larvae | Cohort 1 density (m^-3) | Cohort 1 evasion factor | Cohort 1 adjusted density (m^-3) | Cohort 2 no. larvae | Cohort 2 density (m^-3) | Cohort 2 evasion factor | Cohort 2 adjusted density (m^-3) | Cohort 3 no. larvae | Cohort 3 density (mu-3) | Cohort 3 evasion factor | Cohort 3 adjusted density (m^-3) | Total no. larvae | Total density (m^-3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Bass Harbor | | | | | | | | |
| 3-May-89 | 0 | 0.000 | | 0.000 | 209 | 1.335 | 1.1925 | 1.592 | 0 | 0.000 | - | 0.000 | 209 | 1.335 |
| 3-May-89 | 0 | 0.000 | - | 0.000 | 142 | 0.663 | 1.1925 | 0.791 | 0 | 0.000 | - | 0.000 | 142 | 0.663 |
| 12-May-89 | 0 | 0.000 | - | 0.000 | 16976 | 143.532 | 1.2299 | 176.523 | 0 | 0.000 | - | 0.000 | 16976 | 143.532 |
| 21-May-89 | 0 | 0.000 | - | 0.000 | 654 | 3.340 | 1.2795 | 4.273 | 0 | 0.000 | " | 0.000 | 654 | 3.340 |
| 2 l-May-89 | 0 | 0.000 | - | 0.000 | 1018 | 4.522 | 1.2795 | 5.786 | 0 | 0.000 | - | 0.000 | 1018 | 4.522 |
| 29-May-89 | 0 | 0.000 | - | 0.000 | 58 | 0.350 | 1.3721 | 0.480 | 0 | 0.000 | - | 0.000 | 58 | 0.350 |
| 29-May-89 | 0 | 0.000 | - | 0.000 | 11 | 0.104 | 1.3721 | 0.143 | 0 | 0.000 | - | 0.000 | 11 | 0.104 |
| 8-Jun-89 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | 1.3251 | 0.000 | 0 | 0.000 | - | 0.000 | 0 | 0.000 |
| 8-Jun-89 | 0 | 0.000 | - | 0.000 | 1 | 0.005 | 1.3251 | 0.007 | 0 | 0.000 | - | 0.000 | 1 | 0.005 |
| 13-Jun-89 | 0 | 0.000 | - | 0.000 | 3 | 0.016 | 1.4172 | 0.023 | 2 | 0.013 | 1.34895 | 0.017 | 5 | 0.032 |
| 13-Jun-89 | 0 | 0.000 | - | 0.000 | 2 | 0.013 | 1.4172 | 0.019 | 2 | 0.013 | 1.34895 | 0.018 | 4 | 0.027 |
| 21-Jun-89 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | 1.4431 | 0.000 | 1 | 0.008 | 1.43746 | 0.011 | 1 | 0.008 |
| 21-Jun-89 | 0 | 0.000 | - | 0.000 | 1 | 0.006 | 1.4431 | 0.009 | 2 | 0.013 | 1.43746 | 0.018 | 3 | 0.019 |
| | | | | | | **Fairmount** Island | | | | | | | | |
| 2-May-89 | 0 | 0.000 | - | 0.000 | 1534 | 9.964 | 1.2103 | 12.059 | 0 | 0.000 | - | 0.000 | 1534 | 9.964 |
| 2-May-89 | 0 | 0.000 | - | 0.000 | 1221 | 6.005 | 102103 | 7.267 | 0 | 0.000 | - | 0.000 | 1221 | 6.005 |
| 12-May-89 | 0 | 0.000 | - | 0.000 | 46288 | 365.709 | 1.23595 | 451.998 | 0 | 0.000 | - | 0.000 | 46288 | 365.709 |
| 21-May-89 | 0 | 0.000 | - | 0.000 | 6112 | 44.359 | 1.28415 | 56.964 | 0 | 0.OOO | " | 0.000 | 6112 | 44.359 |
| 29-May-89 | 0 | 0.000 | - | 0.000 | 262 | 1.700 | 1.34131 | 2.280 | 29 | 0.189 | 1.23522 | 0.233 | 291 | 1.889 |

Table B2. Number and density of herring larvae in Prince William Sound in 1989. (Continued)

| | Cohort 1 | | | | Cohort 2 | | | | Cohort 3 | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | no. larvae | density (mu-3) | evasion factor | adjusted density (inn-3) | no. larvae | density (mu-3) | evasion factor | adjusted density (m^-3) | no. larvae | density (m^-3) | evasion factor | adjusted density (inn-3) | no. larvae | density (mu-3) |
| 29-May-89 | o | 0.000 | - | 0.000 | 313 | 2.026 | 1.34131 | 2.717 | 35 | 0.225 | 1.23522 | 0.278 | 348 | 2.251 |
| 7-Jun-89 | 0 | 0.000 | - | 0.000 | 40 | 0.230 | 1.42896 | 0.329 | 136 | 0.771 | 1.27741 | 0.985 | 176 | 1.002 |
| 7-Jun-89 | 0 | 0.000 | - | 0.000 | 58 | 0.384 | 1.42896 | 0.548 | 196 | 1.285 | 1.27741 | 1.641 | 254 | 1.669 |
| 13-Jun-89 | 0 | 0.000 | | 0.000 | 5 | 0.032 | 1.48399 | 0.048 | 5 | 0.032 | 1.31419 | 0.042 | 10 | 0.068 |
| 13-Jun-89 | 1 | 0.007 | - | 0.007 | 8 | 0.056 | 1.48399 | 0.083 | 8 | 0.056 | 1.31419 | 0.074 | 17 | 0.119 |
| 22-Jun-89 | 0 | 0.000 | - | 0.000 | 4 | 0.027 | 1.4001 | 0.037 | 3 | 0.020 | 1.48177 | 0.030 | 7 | 0.047 |
| 22-Jun-89 | 0 | 0.000 | - | 0.000 | 8 | 0.053 | 1.4001 | 0.075 | 6 | 0.040 | 1.48177 | 0.059 | 14 | 0.093 |
| | | | | | | Rocky Bay | | | | | | | | |
| 3-May-89 | 0 | 0.000 | 1.2813 | 0.000 | 2 | 0.007 | 1.18974 | 0.008 | 0 | 0.000 | - | 0.000 | 2 | 0.009 |
| 3-May-89 | 1 | 0.006 | 1.2813 | 0.007 | 3 | 0.017 | 1.18974 | 0.020 | 0 | 0.000 | - | 0.000 | 4 | 0.022 |
| 12-May-89 | 271 | 1.712 | 1.3318 | 2.279 | 26865 | 169.446 | 1.22761 | 208.014 | 0 | 0.000 | | 0.000 | 27136 | 171.158 |
| 21-May-89 | 0 | 0.000 | - | 0.000 | 209 | 1.308 | 1.2608 | 1.649 | 0 | 0.000 | | 0.000 | 209 | 1.308 |
| 21-May-89 | 0 | 0.000 | - | 0.000 | 341 | 1.530 | 1.2608 | 1.930 | 0 | 0.000 | | 0.000 | 341 | 1.530 |
| 30-May-89 | 0 | 0.000 | - | 0.000 | 11 | 0.052 | 1.30454 | 0.068 | 3 | 0.015 | 1.22563 | 0.018 | 14 | 0.068 |
| 30-May-89 | 0 | 0.000 | | 0.000 | 2 | 0.007 | 1.30454 | 0.010 | 0 | 0.000 | 1.22563 | 0.000 | 2 | 0.010 |
| 7-Jun-89 | 0 | 0.000 | - | 0.000 | 11 | 0.113 | 1.46819 | 0.166 | 6 | 0.061 | 1.26011 | 0.077 | 17 | 0.173 |
| 7-Jun-89 | 0 | 0.000 | | 0.000 | 15 | 0.159 | 1.46819 | 0.233 | 8 | 0.085 | 1.26011 | 0.107 | 23 | 0.243 |
| 12-Jun-89 | 0 | 0.000 | - | 0.000 | 1 | 0.009 | 1.47087 | 0.013 | 0 | 0.000 | - | 0.000 | 1 | 0.009 |
| 12-Jun-89 | 0 | 0.000 | | 0.000 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | | 0.000 | 0 | 0.000 |
| 21-Jun-89 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | - | 0.000 | 0 | 0.000 |
| 21-Jun-89 | 0 | 0.000 | | 0.000 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | | 0.000 | 0 | 0.000 |

Table B2. Number and density of herring larvae In Prince William Sound in 1989. (Continued)

| Date | Cohort 1 no. larvae | Cohort 1 density (m^-3) | Cohort 1 evasion factor | Cohort 1 adjusted density (m^-3) | Cohort 2 no. larvae | Cohort 2 density (mu-3) | Cohort 2 evasion factor | Cohort 2 adjusted density (mu-3) | Cohort 3 no. larvae | Cohort 3 density (mu-3) | Cohort 3 evasion factor | Cohort 3 adjusted density (m^-3) | Total no. larvae | Total density (m^-3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Tatitlek Narrows | | | | | | | | |
| 2-May-89 | 16176 | 99.269 | 1.2087 | 119.990 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | - | 0.000 | 16176 | 99.269 |
| 1 l-May-89 | 20752 | 131.395 | 1.2406 | 163.007 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | - | 0.000 | 20752 | 131.395 |
| 20-May-89 | 1667 | 7.076 | 1.3173 | 9.322 | 0 | 0.000 | - | 0.000 | 0 | 0.000 | - | 0.000 | 1667 | 7.076 |
| 30-May-89 | 43 | 0.308 | 1.4540 | 0.448 | 49 | 0.351 | 1.28523 | 0.451 | 0 | 0.000 | - | 0.000 | 92 | 0.659 |
| 30-May-89 | 31 | 0.259 | 1.4540 | 0.376 | 35 | 0.295 | 1.28523 | 0.379 | 0 | 0.000 | - | 0.000 | 66 | 0.554 |
| 7-Jun-89 | 2 | 0.010 | 1.4833 | 0.015 | 4 | 0.020 | 1.39868 | 0.027 | 1 | 0.005 | 1.25809 | 0.006 | 7 | 0.034 |
| 7-Jun-89 | 5 | 0.025 | 1.4833 | 0.038 | 10 | 0.051 | 1.39868 | 0.071 | 2 | 0.013 | 1.25809 | 0.016 | 17 | 0.089 |
| 12-Jun-89 | 17 | 0.068 | 1.2491 | 0.085 | 29 | 0.119 | 1.48399 | 0.176 | 4 | 0.017 | 1.3314 | 0.023 | 50 | 0.204 |
| 12-Jun-89 | 8 | 0.033 | 1.2491 | 0.041 | 14 | 0.058 | 1.48399 | 0.086 | 2 | 0.008 | 1.3314 | 0.011 | 24 | 0.099 |
| 20-Jun-89 | 0 | 0.000 | - | 0.000 | 2 | 0.017 | 1.46128 | 0.025 | 0 | 0.000 | - | 0.000 | 2 | 0.017 |
| 20-Jun-89 | 0 | 0.000 | - | 0.000 | 1 | 0.007 | 1.46128 | 0.010 | 0 | 0.000 | - | 0.000 | 1 | 0.007 |

Notes:

1. Dashes indicate data not available.

# Appendix C. Non-herring Fish Larvae from Prince William Sound

## C1. INTRODUCTION

Little is known of the composition of ichthyoplankton in the waters of Prince William Sound because few studies have included more than one or two stations inside the Sound and all previous plankton surveys in the Gulf of Alaska, except for Kendall and Dunn (1985), have treated ichthyoplankton as a secondary objective. This appendix reports the abundance and taxonomic composition of larval fishes that were captured at four sites in Prince William Sound as part of the herring larval survey. It uses this data to test for possible effects of oil on the abundance of non-herring fish larvae. The working hypothesis is that the two oiled sites (Bass Harbor and Rocky Bay) had lower numbers and diversity than the two control sites (Fairmount Island and Tatitlek Narrows).

## C2. METHODS

Fish larvae were sorted from the formalin-preserved samples taken with the 333 and 505 $\mu$m mesh bongo nets. Fish eggs were not separated from the plankton or identified. Larvae were identified to the species/family level with the use of a laboratory guide (Matarese et al. 1986) and by consultation with J. Marliave (Vancouver Public Aquarium, Vancouver, B.C., Canada) and A. Kendall (Northwest and Alaska Fisheries Center, NMFS, NOAA, Seattle, WA).

## C3. RESULTS AND DISCUSSION

### C3.1 Total Numbers

The plankton samples contained 5,482 non-herring fish larvae distributed among 28 species and 12 families (Table C1). Walleye pollock, Theragra chalcogramma, made up 76,S% of the total number of non-herring fish larvae followed by capelin, Mallotus villosus (8.6%), flathead sole, Hippoglossoides elassodon (4.8910), northern smoothtongue, Leuroglossus schmidtii (3.2%), and starry flounder, Platichthys stellatus (2.0%) (Fig. C1). The remaining 23 species each made up less than 1% of the total number. Except for the ubiquity of walleye pollock, this rank ordering does not resemble any of the recurrent groups found on the continental shelf of Kodiak

Table Cl. Non-herring larvae.

| Date | Site | Microstomas pacificus no. | Microstomas pacificus density (m-3) | Hippoglossoides elassodon no, | Hippoglossoides elassodon density (m3) | Isopsetta isolepis no. | Isopsetta isolepis density (mu-3) | Lepidopsetta bilineata no. | Lepidopsetta bilineata density (mu-3) | Parophrys vetulus no. | Parophrys vetulus density (m-3) | Platichthys stellatus no. | Platichthys stellatus density (m-3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-May-69 | Bass Harbour | - | - | 1 | 0.006 | - | - | - | - | - | - | - | - |
| 12-May-89 | Bass Harbour | - | - | | . | - | - | | | - | - | - | - |
| 21-May-69 | 8sss Harbour | 1 | 0.005 | 8 | 0.041 | - | - | - | - | - | - | 9 | 0.046 |
| 29-May-89 | Bass Harbour | . | . | 1 | 0.006 | - | - | - | - | - | - | . | . |
| 8-Jun-89 | 6sss Harbour | . | . | 1 | 0.005 | - | - | - | - | - | - | - | - |
| 13-Jun-89 | Baas Harbour | - | - | - | | - | - | - | - | - | - | . | . |
| 21-Jun-89 | Bass Harbour | - | - | - | | - | - | - | | - | - | . | . |
| | SUM | 1 | 0.005 | 11 | 0.059 | o | 0.000 | 0 | 0.000 | 0 | 0.000 | 9 | 0.046 |
| 2-May-69 | Fairmount Island | - | - | 1 | 0.005 | - | - | 2 | 0.010 | - | - | 1 | 0.005 |
| 12-May-89 | Fairmount Island | - | - | . | | - | - | - | | - | - | - | - |
| 21-May-89 | Fairmount Island | - | - | | | - | - | | | - | - | - | - |
| 29-May-89 | Fairmount Island | - | - | 1 | 0.006 | - | - | | | - | - | - | - |
| 7-Jun-89 | Fairmount Island | - | - | | | - | - | | | - | - | - | - |
| 13-Jun-89 | Fairmount Island | - | - | | | - | - | - | | - | - | - | - |
| 22-Jun-89 | Fairmount Island | - | - | - | - | - | - | - | - | - | - | - | - |
| | SUM | o | 0.000 | 2 | 0.011 | 0 | 0.000 | 2 | 0.010 | 0 | 0.000 | 1 | 0.005 |
| 3-May-69 | Rocky Bay | - | - | 5 | 0.028 | - | - | 4 | 0.022 | - | - | - | - |
| 12-May-69 | Rocky Bsy | - | - | 48 | 0.303 | 16 | 0.101 | | - | - | - | - | - |
| 21-May-69 | Rocky Bay | - | - | 9 | 0.040 | - | - | - | - | - | - | - | - |
| 30-May-69 | Rocky Bsy | - | - | 4 | 0.019 | - | - | | - | - | - | - | - |
| 7-Jun-89 | Rocky Etsy | - | - | 2 | 0.021 | - | - | | | - | - | - | - |
| 12-Jun-89 | Rocky Bay | - | - | - | - | - | - | - | - | - | - | - | - |
| 21-Jun-89 | Rocky Bay | - | - | - | - | - | - | - | - | - | - | - | - |
| | SUM | o | 0.000 | 66 | 0.411 | 16 | 0.101 | 4 | 0.022 | 0 | 0.000 | 0 | 0.000 |
| 2-May-89 | Tatitlek Narrows | - | - | 96 | 0.589 | - | - | - | - | - | - | 98 | 0.589 |
| 1 l-May-69 | Tatitlek Narrows | - | - | 64 | 0.406 | - | - | 16 | 0.101 | - | - | . | . |
| 20-May-69 | Tatitlek Narrows | - | - | 21 | 0.069 | - | - | 6 | 0.025 | - | - | 1 | 0.004 |
| 30-May-89 | Tatitlek Narrows | - | - | . | | - | - | - | - | - | - | 3 | 0.022 |
| 7-Jun-89 | Tatitlek Narrows | - | - | - | - | - | - | - | | - | - | - | - |
| 12-Jun-89 | Tatitlek Narrows | - | - | - | | - | - | - | - | - | - | - | - |
| 20-Jun-89 | Tatitlek Narrows | - | - | - | - | - | - | | - | *1* | *0.007* | - | - |
| | SUM | o | 0.000 | 181 | 1.064 | 0 | 0.000 | 22 | 0.127 | 1 | *0.007* | 100 | 0.615 |
| | GRAND SUM | 1 | | 262 | | 16 | | 28 | | 1 | | 110 | |

Table Cl. Non-herring larvae.

| Date | Site | Stenobrachius leucopsarus | | Anoplarchus purpurescens | | Lumpenus sagitta | | Stichaeus punctatus | | Xiphister atropurpureus | | Sebastes spp. 1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | density no. (m-3) | | density no. (m-3) | | density no. (mu-3) | | density no. (mu-8) | | density no. (mu-3) | | density no. (m-3) | |
| 3-May-89 | Base Harbour | - | | - | - | - | - | - | - | | - | - | - |
| 12-May-89 | Bass HerbOur | - | - | - | - | - | - | - | - | | | - | - |
| 21-May-89 | Base Harbour | 1 | 0.005 | - | - | - | - | - | - | 1 | 0.005 | - | - |
| 29-May-89 | Baas Harbour | - | - | - | - | - | - | - | - | | | 1 | 0.006 |
| 8-Jun-89 | Base Harbour | | - | - | - | - | - | - | - | | | 4 | 0.021 |
| 13-Jun-89 | Bass Harbour | | - | | | - | - | - | - | | | 28 | 0.186 |
| 21-Jun-89 | Bass Harbour | | - | - | - | - | - | - | - | | | 5 | 0.032 |
| | M | 1 | 0.005 | 0 | 0.000 | 0 | 0.000 | 0 | 0.000 | 1 | 0.005 | 38 | 0.244 |
| 2-May-89 | Fairmount Island | - | | - | - | - | - | - | - | | | - | - |
| 12-May-89 | Fairmount Island | - | | | | - | - | - | - | | | - | - |
| 21-May-89 | Fairmount Island | - | - | | | - | - | - | - | | | - | - |
| 29-May-89 | Fairmount Island | - | | - | - | - | - | - | - | | | - | - |
| 7-Jun-89 | Fairmount Island | - | - | - | - | - | - | - | - | | | - | - |
| 13-Jun-89 | Fairmount Island | - | - | | | - | - | - | - | | | 1 | 0.007 |
| 22-Jun-89 | Fairmount Island | | - | | - | - | - | - | - | | | - | - |
| | SUM | 0 | 0.000 | 0 | 0.000 | 0 | 0.000 | 0 | 0.000 | 0 | 0.000 | 1 | 0.007 |
| 3-May-89 | Rocky Bay | - | - | - | - | - | - | | - | - | | - | - |
| 12-May-89 | Rocky Bay | - | - | - | - | - | - | - | - | - | | - | - |
| 21-May-88 | Rocky say | - | - | 1 | 0.004 | 2 | 0.009 | 2 | 0.008 | | | - | - |
| 30-May-89 | Rocky Bay | - | - | | | - | - | - | | | | - | - |
| 7-Jun-89 | Rocky Bay | 1 | 0.011 | - | - | | - | | - | | | - | - |
| 12-Jun-89 | Rocky Bay | - | | - | - | - | - | - | | | | - | - |
| 21-Jun-89 | Rocky Bay | - | | - | - | | - | - | | | | 1 | 0.009 |
| | SUM | 1 | 0.011 | 1 | 0.004 | 2 | 0.009 | 2 | 0.009 | 0 | 0.000 | 1 | 0.009 |
| 2-May-89 | Tatitlek Narrows | - | | - | - | - | - | - | - | | | - | - |
| 1 l-May-89 | Tatitlek Narrowa | - | - | | - | - | - | - | - | - | | - | - |
| 20-May-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | | | - | - |
| 30-May-89 | Tatitlek Narrowa | | - | - | - | - | - | - | - | - | | - | - |
| 7-Jun-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | | | - | - |
| 12-Jun-89 | Tatitlek Narrows | - | | - | - | - | - | - | - | | | - | - |
| 20-Jun-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | | | 5 | 0.033 |
| | M | 0 | 0.000 | 0 | 0.000 | 0 | 0.000 | 0 | 0.000 | 0 | 0.000 | 5 | 0.033 |
| | GRAND SUM | 2 | | 1 | | 2 | | 2 | | 1 | | 45 | |

Table Cl. Non-herring larvae.

| Date | Site | Sebastes Spp. 2 no. | Sebastes Spp. 2 density (m-3) | Sebastes Spp. 3 no. | Sebastes Spp. 3 density (mu-3) | Artedius fenestralis no. | Artedius fenestralis density (mu-3) | Artedius harringtoni no. | Artedius harringtoni density (m^-3) | Artedius meanyi no. | Artedius meanyi density (m-3) | Clinocottus acuticeps no. | Clinocottus acuticeps density (m-3) | Malacocottus zonurus no. | Malacocottus zonurus density (m^-3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-May-89 | Bass Harbour | - | - | - | - | - | - | - | - | - | - | - | - | | |
| 12-May-89 | Base Harbour | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 21-May-89 | Ssss Harbour | 10.00s | | - | - | - | - | - | - | - | - | - | - | - | - |
| 29-May-89 | Bass Harbour | - | - | - | - | - | - | - | - | 1 | 0.006 | - | - | | |
| 8-Jun-89 | Bass Harbour | - | - | - | - | - | - | - | - | 7 | 0.037 | - | - | | |
| 13-Jun-89 | Bass Harbour | - | - | - | - | 4 | 0.027 | 2 | 0.013 | 7 | 0.046 | - | - | - | - |
| 21-Jun-89 | Base Harbour | 1 | 0.006 | 1 | 0.006 | - | - | - | - | 4 | 0.025 | - | - | - | - |
| | SUM | 2 | 0.011 | 1 | 0.006 | 4 | 0.027 | 2 | 0.013 | 19 | 0.115 | 0 | 0.000 | 0 | 0.000 |
| 2-May-89 | Fairmount Island | - | - | - | - | - | - | - | - | 1 | 0.005 | - | - | 1 | 0.005 |
| 12-May-89 | Fairmount Island | - | - | - | - | - | - | - | - | - | - | - | - | | |
| 21-May-89 | Fairmount Island | 3 | 0.022 | - | - | - | - | - | - | 2 | 0.015 | - | - | - | - |
| 29-May-69 | Fairmount Island | 11 | 0.071 | - | - | 2 | 0.013 | | 0.006 | - | - | - | - | 2 | 0.013 |
| 7-Jun-89 | Fairmount Island | 6 | 0.039 | 1 | 0.007 | - | - | | 0.013 | - | - | - | - | - | - |
| 13-Jun-89 | Fairmount Island | | 0.021 | - | - | - | - | - | - | - | - | - | - | - | - |
| 22-Jun-89 | Fairmount Island | | 0.040 | - | - | - | - | - | - | - | - | - | - | - | - |
| | SUM | 29 | 0.193 | 1 | 0.007 | 2 | 0.013 | 3 | 0.020 | 3 | 0.019 | 0 | 0.000 | 3 | 0.018 |
| 3-May-89 | Rocky Say | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| 12-May+9 | Rocky say | - | - | - | - | - | - | - | - | - | - | - | - | | |
| 21-May-89 | Rocky Say | 1 | 0.004 | - | - | 1 | 0.004 | 1 | 0.004 | 2 | 0.009 | - | - | - | |
| 30-May-89 | Rocky Bay | - | - | - | - | - | - | - | - | 2 | 0.010 | - | - | - | |
| 7-Jun-89 | Rocky Bay | | 0.063 | - | - | - | - | - | - | 3 | 0.032 | 1 | 0.011 | - | |
| 12-Jun-89 | Rocky Say | - | - | - | - | - | - | - | - | 2 | 0.018 | - | - | - | |
| 21-Jun-89 | Rocky Bay | - | - | - | - | - | - | 1 | 0.009 | 3 | 0.028 | - | - | - | |
| | SUM | 7 | 0.0s8 | o | 0.000 | 1 | 0.004 | 2 | 0.014 | 12 | 0.096 | 1 | 0.011 | 0 | 0.000 |
| 2-May-69 | Tatitlek Narrows | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 l-May-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | 16 | 0.101 | - | - | - | - |
| 20-May-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 30-May-89 | Tatitlek Narrows | 3 | 0.022 | - | - | - | - | - | - | - | - | - | - | - | - |
| 7-Jun-89 | Tatitlek Narrowa | - | - | - | - | 2 | 0.010 | 1 | 0.005 | 3 | 0.015 | 1 | 0.005 | | |
| 12-Jun-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 20-Jun-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | SUM | 3 | 0.022 | 0 | 0.000 | 2 | 0.010 | 1 | 0.005 | 19 | 0.116 | 1 | 0.005 | 0 | 0.000 |
| | GRAND SUM | 41 | | 2 | | 9 | | 8 | | 53 | | 2 | | 3 | |

Table CI. Non-herring larvae.

| Date | site | Nautichthys oculofasciatus no. | Nautichthys oculofasciatus density (m^-3) | Radulinus Spp. no. | Radulinus Spp. density (m^-3) | Agonid spp. no. | Agonid spp. density (mu-3) | Ammodytes hexapterus no. | Ammodytes hexapterus density (mu-3) | Leuroglossus schmidti no. | Leuroglossus schmidti density (m^-3) | Bathymaster Spp. no. | Bathymaster Spp. density (m^-3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-May-69 | Bass Harbour | - | | 1 | 0.006 | - | - | 2 | 0.013 | | | - | |
| 12-May-39 | Bass Harbour | - | - | - | - | - | - | - | - | - | - | - | |
| 21-May-69 | Bass Harbour | | - | 1 | 0.005 | 1 | 0.005 | - | - | 2 | 0.010 | 2 | 0.010 |
| 29-May-69 | Bass Harbour | - | - | - | - | - | - | - | - | 4 | 0.024 | 1 | 0.006 |
| 8-Jun-89 | Bass Harbour | - | | - | - | - | - | - | - | 3 | 0.016 | 2 | 0.011 |
| 13-Jun-89 | 6sss Harbour | | | 1 | 0.007 | - | - | - | - | 3 | 0.020 | - | |
| 21-Jun-89 | Bass Harbour | - | | 2 | 0.013 | - | - | - | - | 1 | 0.006 | - | |
| | SUM | 0 | 0.000 | 5 | 0.031 | 1 | 0.005 | 2 | 0.013 | 13 | 0.076 | 5 | 0.027 |
| 2-May-69 | Fairmount Island | | | - | - | 1 | 0.005 | - | - | 9 | 0.452 | - | - |
| 12-May-69 | Fairmount Island | | | - | - | - | - | - | - | | | | |
| 21-May-69 | Fairmount Island | - | | 2 | 0.015 | - | - | - | - | 28 | 0.203 | - | - |
| 29-May-89 | Fairmount Island | | | - | - | - | - | - | - | 14 | 0.091 | - | - |
| 7-Jun-89 | Fairmount Island | | - | - | - | - | - | - | - | 13 | 0.065 | 4 | 0.026 |
| 13-Jun-89 | Fairmount Island | | | - | - | - | - | - | - | | | 1 | 0.007 |
| 22-Jun-89 | Fairmount Island | - | | - | - | - | - | - | - | 3 | 0.020 | 10 | 0.067 |
| | SUM | 0 | 0.000 | 2 | 0.015 | 1 | 0.005 | 0 | 0.000 | 150 | 0.852 | 15 | 0.100 |
| 3-May-69 | Rocky Bay | | | 1 | 0.006 | - | - | - | - | 2 | 0.011 | - | - |
| 12-May-89 | Rocky Bay | - | | - | - | - | - | - | - | | | - | - |
| 21-May-69 | Rocky Bay | - | | 1 | 0.004 | - | - | - | - | | - | - | - |
| 30-May-89 | Rocky Bay | 1 | 0.005 | - | - | - | - | - | - | | | - | - |
| 7-Jun-89 | Rocky Bay | | | 1 | 0.011 | - | - | - | - | | | | |
| 12-Jun-89 | Rocky Bay | - | - | - | - | - | - | - | - | | | | |
| 21-Jun-89 | Rocky Bay | - | | - | - | 1 | 0.009 | - | - | - | | 1 | 0.009 |
| | SUM | 1 | 0.005 | 3 | 0.021 | 1 | 0.009 | 0 | 0.000 | 2 | 0.011 | 1 | 0.009 |
| 2-May-69 | Tatitlek Narrows | - | - | - | - | - | - | - | - | | | - | |
| 11-May-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | | | | |
| 20-May-69 | Tatitlek Narrows | - | | 2 | 0.008 | 1 | 0.004 | 3 | 0.013 | 6 | 0.025 | - | - |
| 30-May-69 | Tatitlek Narrows | - | - | - | - | - | - | - | - | 1 | 0.007 | | |
| 7-Jun-89 | Tatitlek Narrows | - | - | - | - | 1 | 0.005 | - | - | 1 | 0.005 | - | |
| 12-Jun-89 | Tatitlek Narrows | - | - | - | - | - | - | - | - | | - | - | |
| 20-Jun-89 | Tatitlek Narrows | - | - | - | - | 1 | 0.007 | - | - | - | - | 1 | *0.007* |
| | SUM | 0 | 0.000 | 2 | 0.006 | 3 | 0.016 | 3 | 0.013 | 8 | 0.038 | 1 | *0.007* |
| | GRAND SUM | 1 | | 12 | | 6 | | 5 | | 173 | | 22 | |

Table Cl. Non-herring larvae.

| Data | Site | Theragra chalcogramma no. | Theragra chalcogramma density (m-3) | Liparis app. no. | Liparis density (mu-3) | Mallotus spp. no. | Mallotus spp. density (mu-3) |
|---|---|---|---|---|---|---|---|
| 3-May-68 | Bass Harbour | 204 | 1.303 | - | - | - | - |
| 12-May-68 | Bass Harbour | 16 | 0.135 | - | - | - | - |
| 21-May-89 | 6sss Harbour | 17 | 0.087 | - | - | - | - |
| 29-May-69 | 6sss Harbour | 5 | 0.030 | - | - | - | - |
| 8-Jun-89 | Bass Harbour | - | - | - | - | 1 | 0.005 |
| 13-Jun-89 | Bass Harbour | - | - | 1 | 0.007 | 7 | 0.046 |
| 21 -Jun-89 | Bass Harbour | - | - | - | - | 108 | 0.685 |
| | SUM | 242 | 1.555 | 1 | 0.007 | 116 | 0.736 |
| 2-May-88 | Fairmount Island | 661 | 3.251 | - | - | - | - |
| 12-May-68 | Fairmount Island | 80 | 0.632 | - | - | - | - |
| 21-May-89 | Fairmount Island | 9 | 0.065 | 1 | 0.007 | - | - |
| 29-May-89 | Fairmount Island | - | - | - | | - | - |
| 7-Jun-89 | Fairmount Island | - | - | - | - | - | - |
| 13-Jun-89 | Fairmount Island | *12* | *0.084* | 1 | *0.007* | 4 | 0.028 |
| 22-Jun-89 | Fairmount Island | | | | | 14 | 0.083 |
| | SUM | 762 | 4032 | 2 | 0.014 | 18 | 0.121 |
| 3-May-89 | Rocky Bay | 167 | 0.920 | - | - | - | - |
| 12-May-89 | Rocky Bay | 304 | 1.917 | - | | - | - |
| 21-May-89 | Rocky Bay | 26 | 0.117 | - | - | - | - |
| 30-May-89 | Rocky Bay | 5 | 0.024 | - | - | - | - |
| 7-Jun-89 | Rocky Bay | 2 | 0.021 | - | - | - | - |
| 12-Jun-89 | Rocky Bay | | - | 1 | 0.009 | 1 | 0.008 |
| 21-Jun-89 | Rocky Bay | - | - | - | - | 328 | 3.087 |
| | SUM | 504 | 3.000 | 1 | 0.009 | 329 | 3,105 |
| 2-May-69 | Tatitlek Narrows | 1728 | 10.604 | - | | - | - |
| 1 l-May-89 | Tatitlek Narrows | 304 | 1.925 | - | - | - | - |
| 20-May-89 | Tatitlek Narrows | 536 | 2.264 | - | - | - | - |
| 20-May-89 | Tatitlek Narrows | 96 | 0.668 | - | - | - | - |
| 7-Jun-89 | Tatitlek Narrows | 20 | 0.086 | - | - | - | - |
| 12-Jun-89 | Tatitlek Narrows | 2 | 0.006 | - | | 1 | 0.004 |
| 20-Jun-89 | Tatitlek Narrows | | - | - | - | 10 | 0.066 |
| | SUM | 2666 | 15.607 | 0 | 0.000 | 11 | 0.070 |
| | GRAND SUM | 4186 | | 4 | | 474 | |

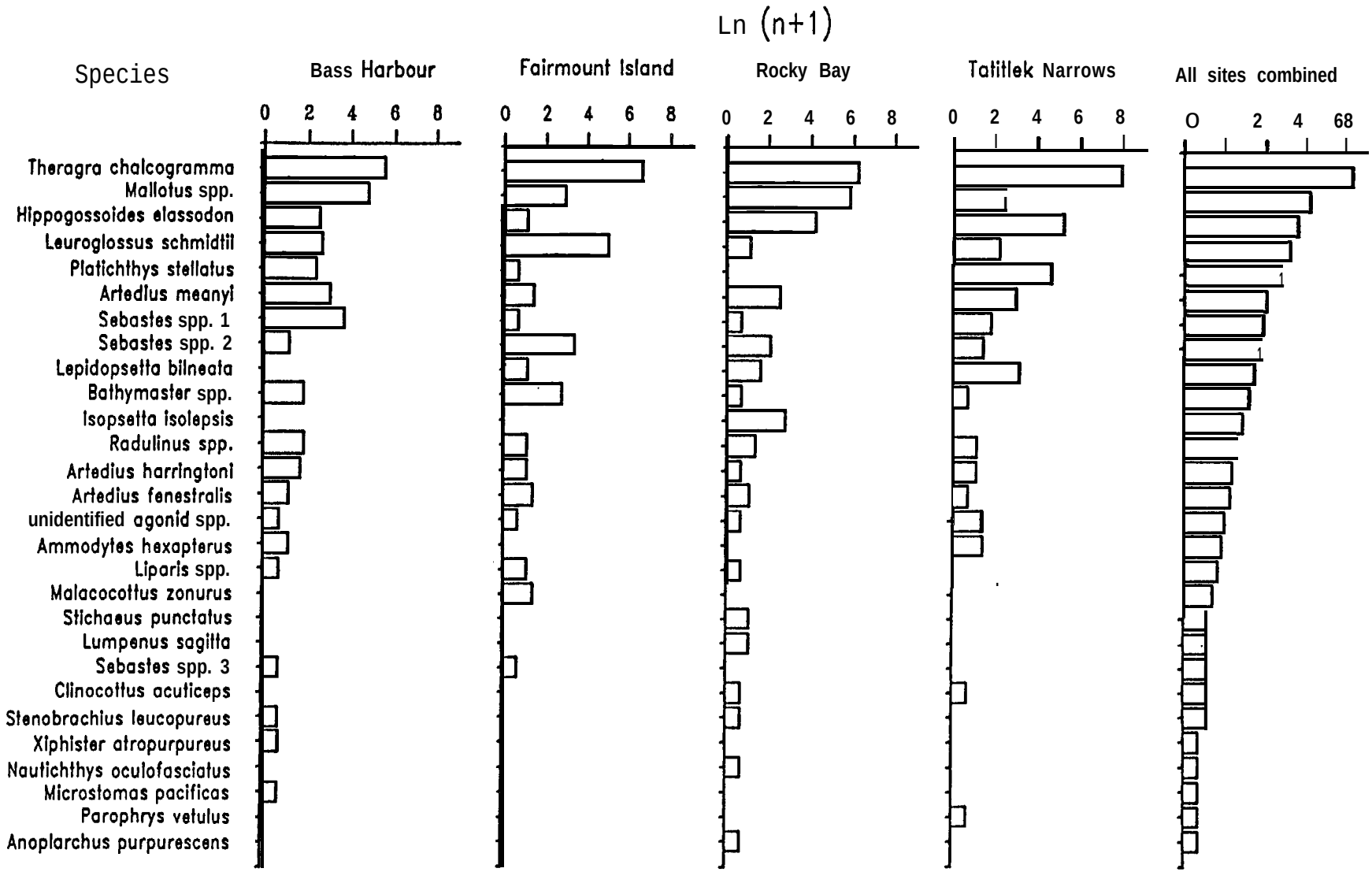


**Figure Cl.** Total number of non-herring fish larvae ranked in decreasing order by site and species.

Island by Kendall and Dunn (1985). The reasons may be related to biophysics factors of Prince William Sound or to the relatively short duration of the sampling period.

Comparisons of total larval abundance between the four sites show that there is little evidence for an oil-related effect on larval abundance or diversity (Fig. Cl). Over half (3,051 or 55.7%) of all non-herring fish larvae were captured at Tatitlek Narrows; Fairmount Island and Rocky Bay had similar numbers: 997 (18.2%) and 960 (17.5%), respectively and Bass Harbor had only 474 (8.6%). However, this rank ordering is due entirely to the fact that pollock made up 88% of all non-herring fish larvae at Tatitlek Narrows. When pollock larvae are removed the rank ordering is: Rocky Bay (456), Tatitlek Narrows (363), Fairmount Island (235) and Bass Harbor (232).

### C3.2 Seasonal Change in Number and Density

Comparisons of changes in total larval numbers with date do not support the hypothesis of an oil effect. Regression analysis showed that the total number of non-herring fish larvae decreased at a daily instantaneous rate of 0.05 d-l ($n = 28$, $r^2 = 0.32$, $P < 0.001$) from May 1 to June 22, but that this trend was due entirely to the loss of pollock larvae from Tatitlek Narrows. If pollock were excluded, there was no significant ($P > 0.05$) correlation between total numbers of larvae and Julian date.

Two species of fish larvae, walleye pollock and northern smoothtongue, were sufficiently abundant to allow a comparison of their seasonal dynamics at each of the four sites. Covariance analysis was used to test for differences between sites in the intercept and slope of linear regressions of ln(density) on Julian date. Zero densities were excluded because they were not true zeros, but probably represented densities too low to be measured by the bongo nets.

The regression model that explained the most variance (? = 0.77, n =19) in walleye pollock densities with all significant (0.0001 <P<0.01) parameters was

| | | | | |
|---|---|---|---|---|
| (cl) | in(N) = | 15.5567 + | 1.6402$g_4$ - | 0.1236D |
| | (SE) | (2.4527) | (0.5135) | (0.0175) |

where N = density (m-3), g4 = dummy variable with a value of 1 for Tatitlek Narrows and O for the other three sites, and D = Julian date. This model shows that pollock larvae were five times more dense at Tatitlek Narrows than at any of the three other sites, but that the daily rate of loss of larvae was the same, 0.12 $d^{-1}$, at all four sites (Fig. C2).

Fig. C3 shows that only the densities of northern smoothtongue larvae from Fairmount Island exhibited a linear trend with date. The best-fitting regression for that site was:

(C2) In(N)= 6.6206- 0.0596D

$r^2$ = 0.96, 0.01<P<0.05

In summary, there is no evidence from the catch curves of walleye pollock or northern smoothtongue to support the hypothesis of an oil effect. The differences between sites in larval numbers and densities were most likely due to the presence or absence of recent hatching events, to the loss of larvae due to natural mortality, and to transport of larvae by water currents.

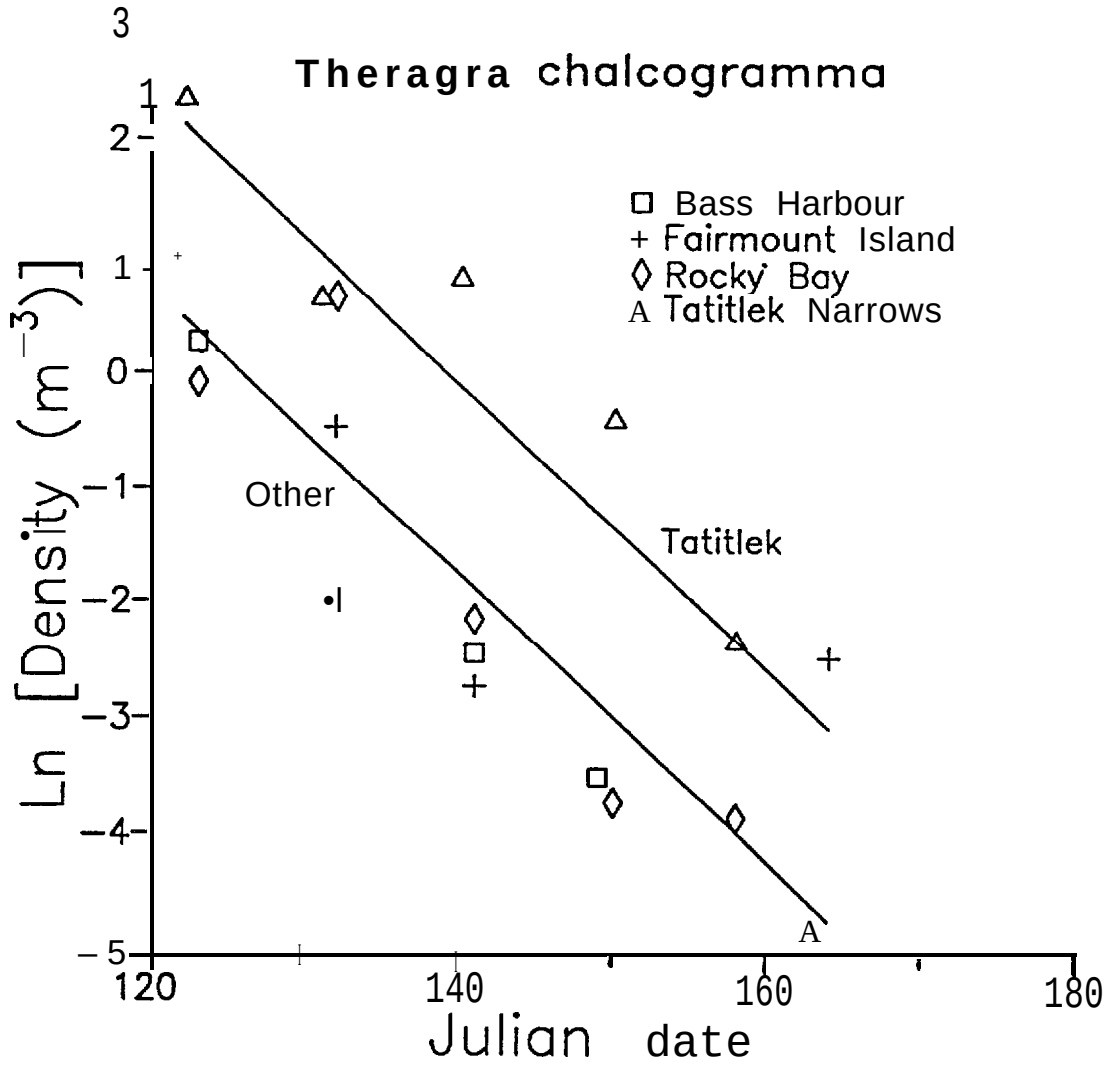


**Figure C2.** **Catch curves of walleye pollock larvae. Highest densities were measured at Tatitlek Narrows, but the rate of loss of density was constant at 0.12 $d^{-1}$ for all four sites. See text and equation (C1)** for detail.

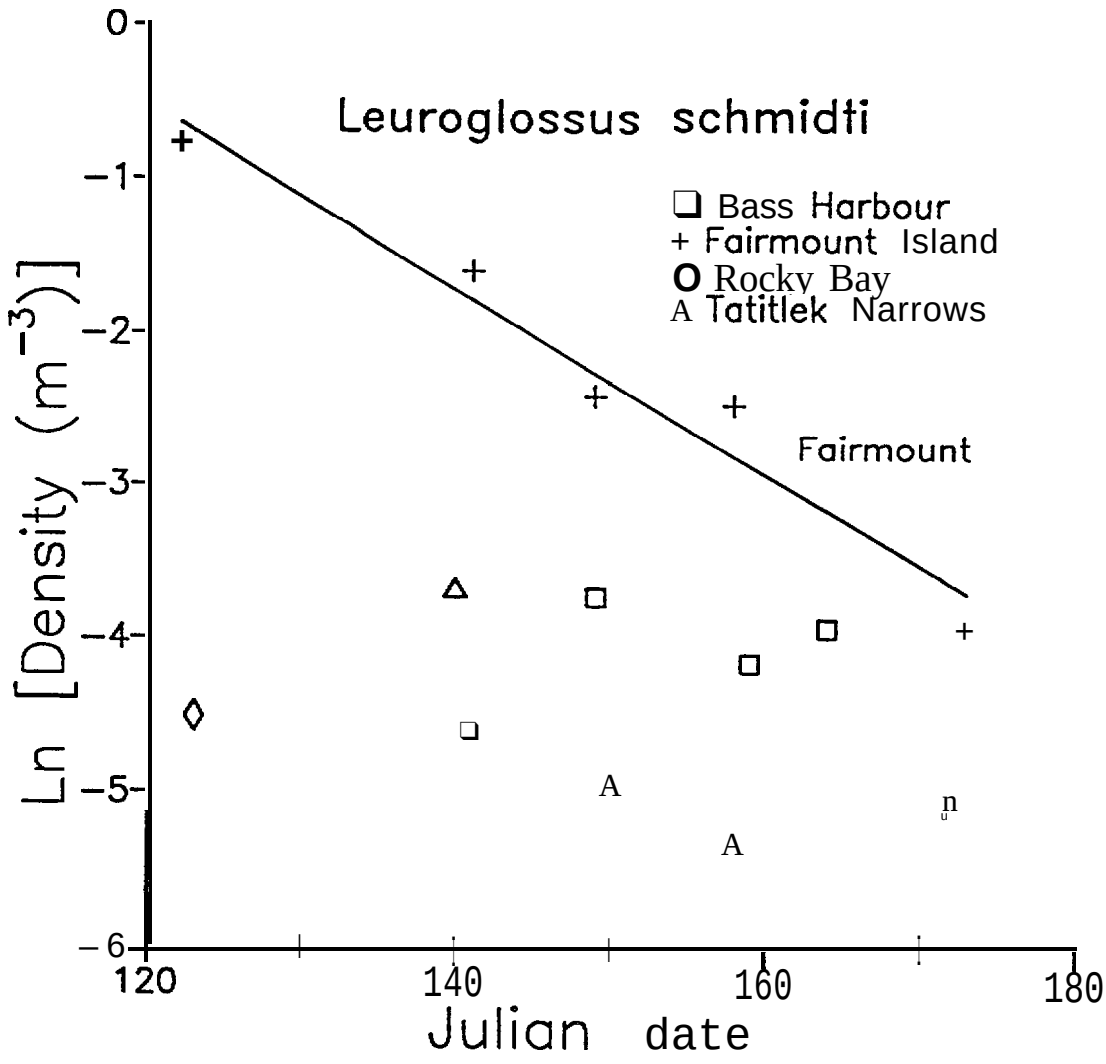


**Figure C3.** **Catch curve of smoothtongue sole larvae. Highest densities were measured at Fairmount Island, and only Fairmount exhibited a significant loss in density with date.**